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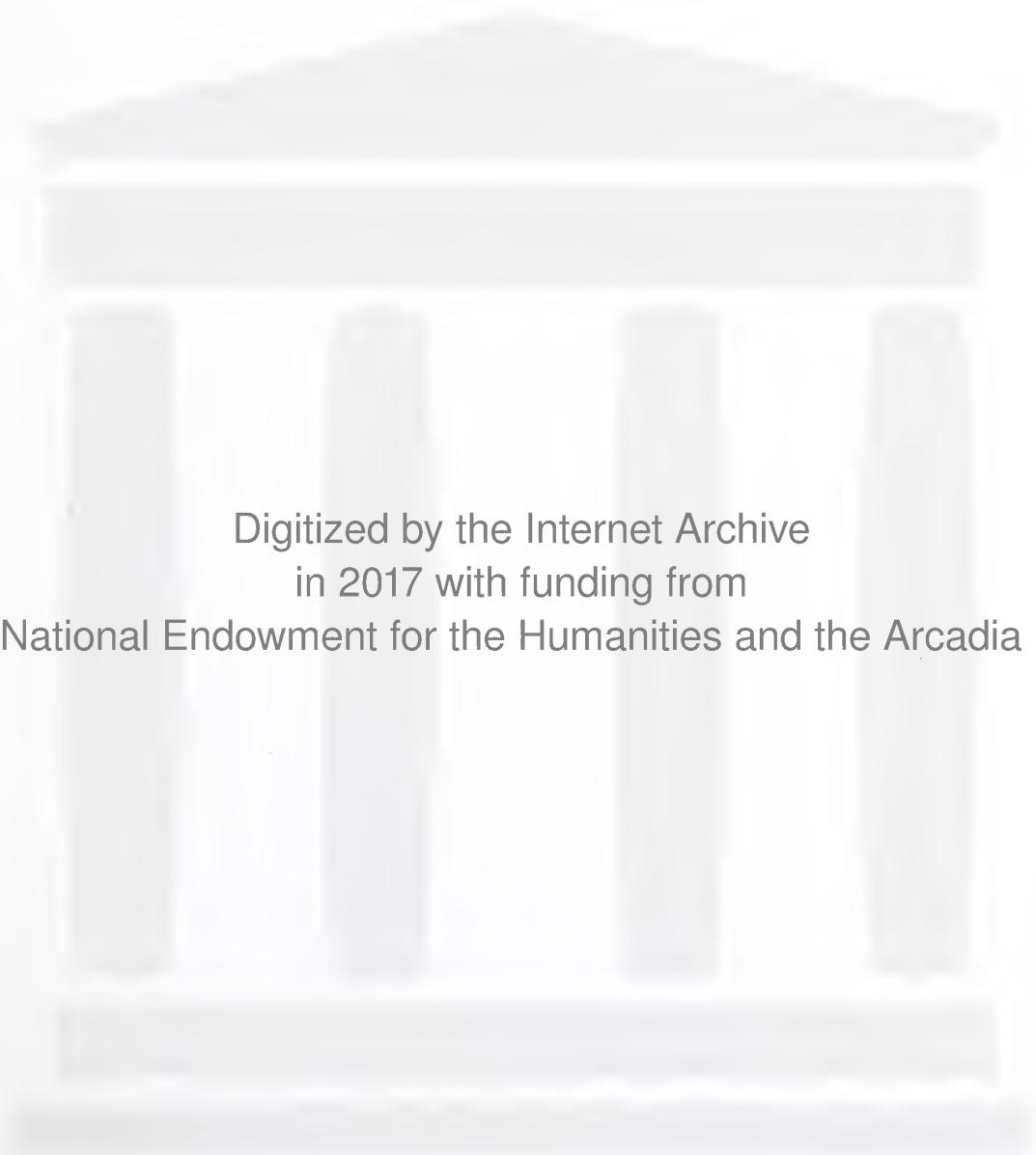

ELICITATION OF THE LATE NASAL AND CUTANEOUS RESPONSES: THE POSSIBLE ROLE OF EOSINOPHILS AND BASOPHILS

by

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ABBREVIATIONS

ABA	allergic bronchopulmonary aspergillosis
band	band neutrophil
CBH	cutaneous basophil hypersensitivity
DSCG	disodium cromoglycate
HPF	high power field (970x)
hr	hour(s)
IgE	immunoglobulin E
IgG	immunoglobulin G
IgG1	immunoglobulin G1
L	liter(s)
LBR	late bronchial response
LCR	late cutaneous response
LNR	late nasal response
LPF	low power field (100x)
min	minute(s)
ml	milliliter(s)
mm	millimeter(s)
mm^3	cubic millimeter(s)
NPT	nasal provocation test(ing)
NR	nasal ratio
p	probability
PNU	protein nitrogen unit(s)
RAST	radioallergosorbent test
Rl	resistance of left nostril
RM	rhinomanometry
Rr	resistance of right nostril
Rt	total resistance
S.D.	standard deviation
seg	segmented neutrophil
WBC	white blood count

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INTRODUCTION

Traditionally, allergic rhinitis and asthma are considered Type I (anaphylactic) reactions (1). The reaction is mediated by histamine, released from the mast cell (or basophil) by the binding of its attached immunoglobulin E (IgE) to the antigen. Histamine, and possibly other vasoactive amines, act pharmacologically producing the clinical symptoms of rhinitis, bronchospasm and/or urticaria. This occurs within minutes, and resolves within 1-2 hours.

However, there is substantial evidence that late asthmatic (2-24) and late nasal (24-28) responses occur, beginning as early as 3 hours (9) and lasting up to one week (2).

It is quite important to differentiate between early and late allergic responses. Nocturnal symptoms may be due to continued provocation (i.e. house dust), or theoretically may be a late response to provocation occurring 12 hours earlier.

In addition to determining the offending stimulus, it is important to select the proper therapeutic agent. In allergic rhinitis, antihistamines have traditionally been the treatment of choice (1). However, these drugs alone are often insufficient. Beclomethasone and other adrenal steriods, applied topically, are quite effective in the treatment of hayfever (28,30-32). Recent evidence (28) suggests that the clinical effect of topical steroids is based on their ability to block late nasal responses.

There is similar evidence demonstrating the effectiveness of topical steroids in asthma (8,10,12,18,33). In addition, disodium

cromoglycate (DSCG) (Intal), has been found effective in both asthma (5,8,9,12,20,34) and allergic rhinitis (35-37).

There are several thoughts as to the etiology of these late responses, which occur in the nasal mucosa, bronchi, and skin (14,24,26, 38-49). Some favor the involvement of immune complexes or an Arthus reaction -- Type III hypersensitivity (7,8,10,12,24,26,28). Others have commented that the late ("delayed") response may be cell-mediated (Type IV) (27,28,40).

Several investigators have examined the role of basophils in late responses (38,41,45). This research was motivated by the model of "cutaneous basophil hypersensitivity" (CBH), a late cutaneous response in guinea pigs (50-52). CBH has a maximal skin response at 24 hours (50,51), and biopsy of the lesion demonstrates a marked accumulation of basophils in the dermis (52).

Basophils have recently been noted in the nasal secretions of subjects with allergic rhinitis (53). Other studies have correlated peripheral basophil counts with allergic symptoms (54-57).

Peripheral eosinophilia is frequently associated with allergic disease (57-59). The presence of eosinophilia in nasal secretions is virtually pathognomonic for allergic rhinitis (1,59-62). Eosinophils have also been found in biopsies of late responses (39,41,45).

The objective of this thesis was to reproducibly elicit late allergic responses, and to investigate further the possible role of basophils and eosinophils in non-immediate hypersensitivity. Late nasal responses were chosen in particular, because bronchial provocation testing may require hospitalization in the event of a severe immediate or

late response. In addition, nasal secretions can be easily obtained and studied.

LATE ALLERGIC RESPONSES

The Late Cutaneous Response (LCR)

In 1934, Jones and Mote (63), in the process of investigating foreign protein sensitization in humans, noted the occurrence of an immediate wheal and flare, followed at 24 hours by a tuberculin type reaction.

In recent years, the LCR has been more thoroughly studied, and is probably better understood than late nasal or bronchial responses. Solley *et al.* (45) describe a pruritic wheal and flare response after antigenic challenge (to pollens and molds), reaching a peak at 15-30 minutes. Over the next hour, the wheal becomes less distinct, merging into the flare. At 90 minutes, the lesion is diffusely edematous and erythematous, but asymptomatic. The lesion remains as such until 4-5 hours post-provocation, when mild pruritis reoccurs. The late response peaks at 6-12 hours, consisting of erythema, warmth, edema, pruritis, and/or tenderness. It is much greater in area than the initial reaction, and often causes greater subjective discomfort. The lesion persists for 24-48 hour macroscopically.

The LCR occurs in a minority of subjects at the usual low intracutaneous doses (26,39), but can be elicited in virtually all subjects at high enough doses (14).

Umemoto *et al.* (46) describe a threshold of an 8 mm immediate response for the occurrence of a late response. However, other investigators (39) have described a late response in nonatopics, occurring independently of the wheal and flare. In studies (42) with detergent industry workers sensitive to Baccillus subtilis enzyme, the

proteinase activity was not necessary for the LCR. Other studies (48) have demonstrated a local relative refractory period of 2-3 weeks, during which time the LCR may occur, but is considerably reduced. This effect is nonspecific, and the mediation unknown.

Much effort has been expended studying the relationship of the wheal and/or flare to the LCR. Brostoff and Roitt (40) ablated the wheal and flare with intradermal chlorpheniramine, but noted the development of a delayed response, maximal at 36 hours. This result could not be confirmed by others (64,65). Green et al. (39) noted over 20% of their LCR's occurred in subjects with a negative immediate response. Umemoto et al. (46) ablated only the flare response (mediated by local sensory nerves), and found no change in the LCR.

Of particular interest is the observation that prednisone inhibits the late response (24,44), but has no effect on the wheal and flare (66).

Many investigators (24,26,38,39,41,45,49) have biopsied the LCR, studying the role of leukocytes, immunoglobulins, complement, and fibrin (see Table I). The infiltrate varies from "scanty" (24) to "intense" (39), and consists of a mixed variety of cells -- mainly neutrophils, lymphocytes and eosinophils. Only one study (24) has demonstrated IgG, and two studies have demonstrated bound complement (24,26).

Recently, De Shazo et al. (49) demonstrated fibrin to be the distinguishing feature between those sites with a positive LCR and a positive wheal-negative LCR control; as the cellular infiltrate was qualitatively the same in both groups (38).

The varying results above have suggested multiple etiologies of the LCR, and raised many questions about the function of each of the possible

TABLE I

Biopsies of the LCR

Study	Cellularity	Dominant Cell	Other Important Cells	Time of Biopsy	IgG	C	Fibrin
Green et al. (39)	"intense" (mild when neg. wheal and flare)	eosinophil	mononuclear	48 hr	ND	ND	ND
Felarca and Lowell (41)	ND	neutrophil	eosinophil basophil	24 hr (window)	ND	ND	ND
McCarthy and Pepys (24)	"scanty or moderate"	mononuclear	eosinophil	2 hr	+	+	ND
Taylor and Shivalkar (26)	ND	neutrophil mononuclear	eosinophil	6-24 hr	ND	+	ND
Solley et al. (45)	moderate	mononuclear (lymphocyte)	eosinophil neutrophil basophil	7-8 hr	-	±	ND
Richerson et al. (38)	variable	mononuclear	neutrophil eosinophil	24 hr	ND	ND	-
Dolovich et al. (43)	ND	eosinophil	neutrophil mononuclear	6 hr	-	-	-
De Shazo et al. (49)	modest	lymphocyte neutrophil	± basophil ± eosinophil	6 hr	-	-	+

Key: IgG immunoglobulin G
C complement
+ frequently found
- not found
± infrequently found
ND not done or not described

participants. The function of the eosinophil is especially intriguing. Eosinophils have also been found in biopsies of the wheal and flare, as early as 15 minutes after antigen challenge (67). It has been suggested (43) that eosinophils are attracted to the site of the LCR, and act similarly to neutrophils in the Arthus reaction.

Other investigators (24,26) feel that the LCR is an Arthus reaction, based on the finding of IgG and/or bound complement in skin biopsies (see above). It was considered that previous hyposensitization may have resulted in the formation of precipitins capable of producing immune complexes, but no correlation between such therapy and the LCR was found (26).

The LCR has also been thought to be a Type IV reaction. It is capable of transfer by peripheral leukocytes (68), and can occur at 36-72 hours (40). Also, positive leukocyte stimulation (27,40) and macrophage migration-inhibition tests (40) have been found in some patients with late responses.

More recent research focuses on the role of mediators and immunoglobulins, specifically IgE. The LCR can be transferred with serum (Prausnitz-Kustner) (43,45); but not with heated serum, or serum following treatment with anti-IgE immunoabsorbent (45). A dual response (wheal and flare followed by LCR) can be elicited by antiserum to human myeloma IgE (43). This response seems identical to the antigen-induced response, and is also inhibited by prednisone (44). Excessive quantities of IgE myeloma protein can inhibit both the immediate and LCR (45).

But the mediation of the LCR is still quite obscure. Histamine alone cannot mediate the LCR, which occurs on the presence of antihistamines (40);

and intradermal injection of histamine produced a significant wheal and flare, but no LCR (45,69). Similar injections with bradykinin or a combination of bradykinin and histamine could not produce the LCR (45,69).

However, the injection of Compound 48/80, causing degranulation of mast cells, is effective in producing a response similar to the LCR (45). Even more interesting is the finding that intradermal injection of kallikrein causes a wheal and flare, followed by a superficial, erythematous, tender nonpruritic dermal infiltration peaking at 5-24 hours, and lasting for up to 48 hours (69). This response was even greater in patients with hereditary angioneurotic edema (70).

One can speculate on a possible explanation linking the appearance of complement (24,26) and fibrin deposits (49), with the effects of kallikrein. The activation of Hageman factor (XII) can initiate the coagulation system (leading to fibrin production), fibrinolytic system (leading to plasmin, and activation of the complement system), and kinin system (leading to kallikrein production) (1). Quite possibly, all of these systems may be involved in the LCR, but not simultaneously; thus the great discrepancy in data described above. It would be interesting to try and block the LCR with kallikrein inhibitor.

Late Responses in the Upper Respiratory Tract

Before discussing the late nasal response (LNR), it is necessary to introduce two important technical methods for eliciting and assessing the existence of such a response. More difficult than intradermal injections, nasal provocation testing (NPT) requires a well-defined methodology to properly produce an immediate and/or LNR. The technique of rhinomanometry (RM) is often used to define the existence of a nasal response.

Rhinomanometry. The technique of RM has evolved from crude clinical methods, to sophisticated electronic gadgetry. The first methods were qualitative, comparing one nostril to the other, by determining the condensation of moisture on a mirror (71). Similarly, the pitch of the tone given off during expiration through one nostril (72), has provided the otolaryngologist with a qualitative assessment of nasal obstruction.

Another noninvasive method was devised by Taylor et al. (73). Using a Wright flow meter, they assessed the amount of nasal obstruction by determining the ratio of the nasal peak flow to the oral peak flow. Their results correlated closely to those of standard RM.

Rhinomanometric techniques can be divided into two broad categories, anterior and posterior. Anterior techniques are generally less invasive, involving the use of exterior, or at most nasal intubation. The pressure difference between the nasopharynx and ambient pressure is recorded in one nostril, while flow is recorded in the other (74). As there is no flow in one side, its external pressure is equal to that of the nasopharynx. (Pressure = flow X resistance. If the flow is zero, then the pressure change through that region is also zero.)

The posterior methods require intubation of the posterior oropharynx or nasopharynx, to determine the difference between ambient pressure and the mesopharynx (74). This method can be more difficult, as subjects must relax the soft palate to produce undistorted recording (75).

Both types of methods have been found satisfactory (74,76). Anterior methods are more sensitive (76), but probably less specific (74). However, they are much more tolerable for the subject. Anterior methods measure each nostril individually, but calculation of the total resistance is not as accurate as direct measurement by posterior methods.

All rhinomanometric methods are based on the assumption of a linear relationship between pressure and flow, or constant resistance. This has been demonstrated to be valid at low flow rates (less than 12 L/min), but nonlinear at progressively higher rates (29,77,78).

Nasal provocation testing. Often the allergic patient describes a clinical history that cannot be confirmed by intradermal testing, or vice versa (79). This often results in confusion about whether or not to treat the allergen in question. A more accurate method is directly testing the end organ, by means of NPT.

The idea is not new, first utilized by Blackley in 1873 (80). Dry pollen was used in the early studies. The usual technique was spraying, but Blumstein (79) devised the unique method of allowing the subject to inhale the pollen from the blunt end of a toothpick. If the patient's usual rhinitic symptoms occurred (sneezing, rhinorrhea, and/or obstruction), the test was considered positive. He found "the dry pollen nasal test" specific in all but 7% of the cases. It corroborated the histories and skin tests in 86% of the cases.

However, the test required greater reproducibility. Connell (81) devised the first quantitative method for NPT. He was able to deliver a fixed number of pollen grains per minute, to reproduce environmental conditions of seasonal rhinitis.

More recent studies have utilized sophisticated equipment, including delivery of pollen via a compressor-powered atomizer (82). The air flow was measured by a rotameter, and duration of flow by a timing unit. This operated a solenoid, which controlled the compressor.

The role of NPT today is controversial. There is a significant lack of conformity between skin and nasal testing (60,83). One study (83) found an overall agreement of 77%. It also seems that some allergens yield better correlations than others (grass pollen, house dust mite) (60).

The emergence of the radioallergosorbent test (RAST) (84), which measures the quantity of specific IgE, is one factor in the decreased use of NPT. Eriksson (85) found a 100% agreement with the NPT, utilizing a combination of the case history, skin testing, and RAST. The correlation between RAST and NPT was only 77%, and the correlation between NPT and a combination of the case history and skin testing was 90%. Another study (86) found no clear positive quantitative correlation between skin testing and NPT; suggesting that local IgE production is of primary importance. In light of the above, NPT still seems to be a reasonably safe, quick, and inexpensive method of determining the significance of clinical allergy to a specific antigen.

The late nasal response. In 1934, Simon and Rackemann (87) noted the symptoms of nasal obstruction, thin serous discharge and sneezing, following the instillation of guinea pig serum via nasal packing (in sensitized individuals). Occasionally, this response occurred at 10-15 minutes (after provocation), but usually it began within from 6-24 hours, and lasted from 2-6 days.

Slavin et al. (25) repeated the above study with similar results. Also, using nasal packs saturated with ragweed pollen extract, there was no immediate response (in nonatopics with induced sensitivity). But rhinorrhea and mild congestion occurred at 6 hours, increasing by 24 hours, and disappearing by 48 hours. In contrast, nasal insufflation of ragweed

(in naturally sensitive subjects) produced immediate symptoms, which disappeared within an hour, without recurrence.

Pelikan (28) describes three different types of nasal response: immediate, late (6-10 hours), and delayed (after 24 hours). The immediate response is characterized by severe rhinorrhea and sneezing, with moderate obstruction. The late response consists of severe obstruction, with only slight rhinorrhea and sneezing. The delayed response is similar to the late, except for even less sneezing and rhinorrhea.

There is some controversy over the frequency of occurrence of the LNR. Various studies have recorded from 0% to 60% late responses, in subjects known to be sensitive to the allergen (see Table II)(24,26,28, 29,30).

The correlation between the LCR and the LNR is high in those studies (24,26) reporting a high frequency of LNR. This has led these investigators to postulate a common mechanism.

It is also interesting that previous tonsillectomy is associated with an increased frequency of LNR (26). This may be secondary to the tonsillectomy itself, or to an increased incidence of tonsillectomy in children with severe, prolonged nasal allergy.

The etiology of the LNR is probably more confusing than that of the LCR. Most investigators believe the two responses are intimately related. Several groups (24,26,28) have implicated the Arthus reaction (Type III hypersensitivity), but the evidence is totally circumstantial; based on findings in skin biopsies in these individuals (see previous section). Other investigators (27,28) claim the LNR to be a delayed-type hypersensitivity (Type IV).

TABLE II

Frequency of Occurrence of Late Nasal and Cutaneous Responses

Study	Frequency of LNR	Frequency of LCR	Correlation (% of subjects with neither or both late responses)
McCarthy and Pepys (24)	60%	67%	93%
Taylor and Shivalkar (26)	37%	30%	81%
Pelikan (28)	3%	ND	ND
Schumacher et al. (29)	16%	ND	ND
Richerson et al. (38)	0%	75%	25%

A "priming effect" was described by Connell (88) in an elaborate series of experiments in 1968, prior to most of the work on late allergic responses. He noted that one-hour nasal challenges (using his method described in previous section) daily, for four days, decreased the nasal threshold to provocation more than five-fold. This effect was readily reversed after several days without challenge. Only the nostril challenged was "primed", and the effect was nonspecific -- priming with one antigen facilitated challenge with another (89). If challenged for four consecutive weeks, more than five weeks were required until the subject's threshold returned to baseline.

Nasal biopsies of "primed" subjects revealed a marked histiocytosis, a dense infiltrate of foamy cells, and many eosinophils (90). In addition, there was destruction of the basement membrane.

Because of the nonspecificity of priming, household allergens of little importance during the majority of the year will cause clinical (often nocturnal) symptoms in the patient during a pollen season (91). Repeated provocation, leading to mucosal change and a decreased threshold (followed by a cascade of more challenge and more damage), could be a cause of late nasal (and bronchial) responses to inhalant allergens. However, it is difficult to explain the occurrence of late responses following a single provocation, and the priming effect does not apply to cutaneous responses.

Taylor and Shivalkar (86) report a local desensitization effect, contrary to Connell's results. They demonstrated an increase in threshold dose following repetitive challenge.

Despite the low incidence of the LNR in some studies, it may be a very important clinical phenomenon. One patient has been described with

a typical syndrome of seasonal rhinitis, but negative immediate responses to skin and nasal challenge with pollens (27). However, he did have late responses at 24-48 hours following provocation with tree pollens. This patient had no specific IgE or IgG, but his lymphocytes could be stimulated specifically in vitro by pollen extract. The lack of specific IgE is consistent with previous studies (85) on the correlation between RAST and NPT. The significance of the positive lymphocyte stimulation test is unclear.

Hay fever sufferers are much less symptomatic at the beginning of a pollen season, than at the end (91). In fact, the pollen count may be near zero at the end of the season, and the patient will be more symptomatic than at high pollen counts at the beginning of the season (26). This clinical finding is consistent with Connell's priming effect (91), although others attribute it to a prolonged Type III reaction (26).

Topical corticosteroids are quite effective in allergic rhinitis (31,32), despite their lack of effectiveness on the immediate nasal response (92,93). One study (30) demonstrated an almost total ablatement of the LNR with beclomethasone. Also noted was a significant reduction in the immediate nasal response with topical steroids, not previously demonstrated.

DSCG also seems to be effective in protecting against nasal provocation (35,37), although there is conflicting evidence (94) on its usefulness in allergic rhinitis. DSCG is quite effective in blocking the late bronchial response (5,8,9,12,20), and is often used clinically for asthma.

Histology of nasal allergy. The examination of nasal secretions is an important step in the diagnosis of allergic rhinitis (1). The

predominant cells in nasal secretions are epithelial in origin, although leukocytes are frequent in nasal specimens collected by blowing ("blown") (53).

Many clinicians and investigators have examined nasal smears for eosinophils, using both swabbed and blown specimens. 50-90% of individuals with allergic rhinitis have significant nasal eosinophilia (59,61,62), compared to only 5% of control subjects (59). Nasal eosinophilia tends to decrease following hyposensitization (62). Eosinophils can sometimes be seen in nasal smears after NPT, usually associated with a positive clinical response (60).

Mast cells have also been found in nasal secretions. This was first noted in 1959 by Bryan and Bryan (95,96). Mast cells can be found in 80-98% of nasal smears from adults with symptomatic allergic rhinitis (53,61). The mast cells are less frequent in children (61), and are not found in blown specimens (53). They are associated with clinical symptoms of allergy, and disappear following treatment with nasal dexamethasone (53).

Basophils have also been noted in blown nasal secretions, but are absent in scraped specimens (53). Like mast cells, they are only present in symptomatic allergic subjects.

In biopsies of the nasal mucosa following provocation, eosinophils are the predominant cell (25,90). Eosinophils are still present at 24 hours after provocation, even in the absence of clinical symptoms (25). Following several days of provocation, Connell noted eosinophils, a dense infiltrate of large foamy cells, and destruction of the basement membrane (90). The clinical significance of these findings is unclear (91).

In subjects with induced ragweed sensitivity, biopsies 6 hours post-provocation were normal, despite the presence of rhinorrhea and congestion (25). Many mononuclear cells were seen at 24 hours, while nasal smears revealed neutrophils and mononuclear cells. These results may not be applicable because of the artificially induced nasal sensitiviy.

Late Responses in the Lower Respiratory Tract

Nocturnal asthma. Salter (97) was the first to recognize the importance of nocturnal asthma. The etiology of this syndrome is not clear. Some have attributed it to variations in the levels of adrenal steroids, noting a decrease in the urinary excretion of 17-hydroxycorticosteroids coincident with an exacerbation of bronchial obstruction (98). However, nocturnal cortisol infusions were ineffective in preventing a fall in peak expiratory flow rate (99).

There may be a decrease in sympathetic tone at night, as suggested by a fall in urinary catecholamine excretion (100). Some workers (101) have found the bronchi to have a lower threshold to provocation at approximately 11 P.M. Yet others (102) have related the decrease in flow rate to sleep. Changes in the daily bronchial cycle occur within 36 hours of modification of the sleep cycle. It seems unlikely that a metabolic cycle could change that rapidly.

A related finding is that positive skin tests are more easily elicited in the evening, than at other times of the day (103).

The late bronchial response. The late bronchial response (LBR), or late asthmatic reaction (24), was first noted by Herxheimer (23) in 1952. It begins within 4-10 hours after bronchial provocation, and can last

from 30-70 hours, longer responses occurring in more severe reactions. The peak reduction in flow rate occurs at more than 8 hours, with the mean peak at 17 hours post-provocation (24). The late bronchial response (like the LNR), tends to produce greater obstruction than the preceding immediate response (17,24).

Some subjects experience a systemic reaction (5,16,24) associated with the LBR; consisting of fever, malaise, leukocytosis, and eosinophilia (16). However, the systemic reaction may be unrelated to any allergic response, but a product of the antigens used; as it has been noted in control subjects without accompanying respiratory symptoms (104).

The LBR occurs frequently, in 3-73% of patients tested (2,6,10,14, 17), sometimes occurring in the absence of an immediate response (5,7). It is more common in more sensitive patients (14); as determined by skin testing, or antigen-specific IgE levels. An increased frequency of late responses was also noted in patients with frequent asthmatic attacks during the preceding year (17).

Some studies (16,21) have documented recurrent nocturnal asthma following a single provocation, in one case (21) lasting up to five subsequent nights. Another study (2) has recorded a LBR lasting up to one week.

The LBR can be elicited by many agents. These include house dust (3,7,8), ragweed pollen (14), grass pollen (19), Aspergillus fumigatus (5,24), Aspergillus niger (15), avian precipitins (5), tolyene di-isocyanate (21), cantharidine beetle (18), Prosopis juliflora (a perennial Indian tree) pollen (20), and piperazine dihydrochloride (9).

The late response to Aspergillus fumigatus has been extensively studied because of its relationship to allergic bronchopulmonary

aspergillosis (ABA) (5,24). As described above, there are often systemic symptoms associated with the LBR. This is particularly common following provocation with A. fumigatus (24). Also, crepitant rales are sometimes noted, in addition to the usual obstructive changes (24). This seems to indicate an alveolar process, distinct from the bronchial pathology which occurs following provocation with most allergens.

Treatment of the LBR is more difficult than the preceding immediate response. Bronchodilators are often ineffective (17,24). DSCG seems to be useful in provocation studies (5,8,12,20,34), although there are conflicting reports (18,105).

Corticosteriods are quite effective in preventing the LBR. Oral prednisone does not prevent the immediate response, but did suppress the expected LBR (24). Inhaled beclomethasone (12) or prednisone (8) both inhibit the LBR, without effect on the immediate response. In one case (18), one week of prior treatment with prednisone suppressed the LBR, while treatment with DSCG failed. Beclomethasone seems to be especially useful, as there is no evidence of adrenal suppression when used topically (by inhalation) (33).

The mechanism of the LBR is as obscure as that of the LNR. It has been suggested (17) that the LBR may be due to mucosal edema of the small airways, and viscous secretions. This would result in early expiratory airway closure and gas trapping. Overinflation could thus occur without changes in the peak expiratory flow, more a measurement of large airway resistance. The LNR, characterized mainly by obstruction (28), probably also is due to mucosal edema. The relative ineffectiveness of bronchodilators (17,24) also suggests a "nonbronchospastic" mechanism for the LBR.

There is some evidence that IgE is involved in the LBR. ABA is associated with high serum concentrations of IgE to Aspergillus fumigatus (106). The LBR to ragweed is more likely to occur in subjects with a high titer of IgE to ragweed antigen E (14).

The Arthus reaction has frequently been implicated in the pathogenesis of the LBR (5,8,24). Unlike the LNR, the LBR is sometimes associated with a systemic response, which is thought to be further evidence of the Arthus reaction (24). However, complement levels remain unchanged (16), and the systemic reaction seems limited to late responses induced by Aspergillus or avian precipitins (5,24). In provocation with house dust extract, there was no evidence of a systemic (fever, leukocytosis) or alveolar (crepitations, radiographic changes) response accompanying the LBR, in 36 patients (8,10).

Possibly, the LBR (and LNR) is due to mediators released at the time of the immediate response (10). This would explain the effectiveness of DSCG, which prevents mediator release from mast cells and basophils. The mediator(s) involved may be long-acting (7), or may be the result of an ongoing immune process. It would be useful to determine if DSCG can prevent late responses if given at a time after the occurrence of the immediate response.

Changes in the Peripheral Eosinophil Count in Allergic Diseases

Peripheral eosinophilia is a hallmark of allergic disease (58). The exact role of these leukocytes in allergy is less well-defined. Eosinophil counts are increased during the attack stage (during an asthmatic attack) of bronchial asthma (57). These individuals also had a greater number of

vacuolated eosinophils, first noted by Connell (107), signifying phagocytosis of antigen-antibody complexes (108).

Increases in the eosinophil count occur immediately following bronchial provocation (24,109). However, the eosinophilia does not correlate with the occurrence of an immediate bronchial response (109). The maximum increase in eosinophil counts occurs at 6 hours after provocation (24) and remains elevated until at least 24 hours after provocation. This was accompanied by leukocytosis, and a late systemic reaction (see previous section).

Peripheral eosinophil counts are not useful in the diagnosis of allergic rhinitis (59), especially compared to examination of the nasal smear.

Changes in the Peripheral Basophil Count in Allergic Diseases

The study of basophils in allergy is a recent phenomenon, probably spurred by the guinea pig model of CBH (50-52) (see next section). There is a significant increase in the basophil count in ragweed-sensitive hay fever sufferers during ragweed season (56). It is also interesting that nonatopic subjects have a significant, but much smaller, elevation of basophils during this time. This may be due to a nonspecific physiologic response to foreign antigens, occurring in normal individuals (56); or it may be related to other environmental factors (changes in temperature, humidity, etc.).

Other studies (54,57) have demonstrated an increase in peripheral basophils prior (within 18 hours) to an asthmatic attack, in the "pre-attack stage". Basophil counts greater than 65/mm^3 were always associated with an asthmatic attack (54).

Basophils have been found in the sputa of patients during an asthmatic attack (110), which were absent during remission periods. Provocation studies (55), with intravenous injection of allergen, have demonstrated a decrease in peripheral basophils, associated with degranulation. These studies seem to indicate a migration of basophils to affected target organs, followed by degranulation and release of mediators.

Cutaneous Basophil Hypersensitivity

Cutaneous basophil hypersensitivity (CBH) is an important animal model of non-immediate hypersensitivity reactions (111,112). Dvorak (52) described the CBH reaction as circumscribed erythematous non-indurated macules. These first appear at 6 hours, peak at 24 hours, and are significantly decreased by 48 hours.

Jones and Mote (63) produced a similar lesion by inoculating humans with rabbit serum. Raffel and Newel (113) coined the term "Jones-Mote reaction" to distinguish these skin reactions, produced transiently after immunization alone or with incomplete Freund's adjuvant, from delayed-type hypersensitivity (DTH) reactions. CBH wanes at the time circulating antibody appears, whereas DTH persists, increasing with time.

Antigens which produce only DTH cannot produce CBH; and those that only stimulate antibody formation do result in a CBH response (51). Carageenan has no effect on CBH, but markedly decreases the tuberculin reaction, an example of DTH (51). High doses of intravenous ovalbumin, yielding tolerance for DTH, are capable of producing CBH and subsequent formation of IgG1 (in guinea pigs) (114). This antibody is homocytotropic, similar to IgE in man (115).

Early studies demonstrated that CBH (116) and Jones-Mote reactions (117) could be passively transferred with cells from draining lymph nodes, but not with serum. However, Askenase (118) demonstrated that the transfer of CBH with immune sera was possible, although the specific factor was still obscure.

T cells can initiate CBH (119), and anti-T cell (120) or anti-lymphocyte (51) serum inhibits CBH. B cells can also initiate CBH (119), confusing this picture even more.

Since CBH reactions occur transiently during a period of little or no specific antibody (51), it was considered that antibody might inhibit CBH. Following immunization with doses of antigens -- dinitrochlorobenzene (116) or vaccinia virus (121) -- insufficient to produce an antibody response, the CBH reaction can be elicited indefinitely. Large doses of passive antiserum do not prevent induction of CBH, but only partially inhibit its expression (122). This effect is nonspecific, and is probably a result of vascular damage by antigen-antibody complexes.

Microscopic examination of CBH reactions reveals a mixed infiltrate, dominated by mononuclear cells and basophils (52,122). 32-44% of the cells were basophils, and mononuclear cells comprised 55-65% of the infiltrate. Scattered neutrophils (3%) and eosinophils (2%) were also seen.

Basophils were often noted in close association with macrophages (52). In fact, many sections seem to demonstrate a phagocytosis of basophils by the macrophages. The explanation for this is unclear.

Unlike the LCR (49), there is no evidence of fibrin deposition in CBH reactions (123). Fibrin accumulation is prominent in DTH reactions, and is probably responsible for the associated induration.

It has recently been demonstrated (124) that basophils are not restricted to CBH reactions. Basophils were found in more than 50% of strongly-positive tuberculin reactions (DTH). This basophilic infiltrate can also be found in human tuberculin reactions (112).

There are several non-immediate immune responses in guinea pigs. CBH is described above. Colvin et al. (122) also describe the "late reaction". These reactions are elicited at 4-6 weeks after immunization, producing a tensely edematous lesion, peaking at 15-30 minutes. Within 4-6 hours the edema diminishes, becoming a moderately hemorrhagic lesion. By 24 hours, only a slightly raised, pale red area remains; which totally disappears by 96 hours.

Evaluation of this reaction suggests that there are three sequential but overlapping phases (122). The first, cutaneous anaphylaxis, is similar to the wheal and flare. The second component is an Arthus reaction, with venous thrombosis, erythrocyte extravasation, and a granulocytic infiltrate. The third phase is a weak CBH reaction.

It is significant that a chronological link is found between the Arthus reaction and CBH. Many observers have attributed late allergic reactions to Type III hypersensitivity, yet others have found no evidence of immune complex disease (see previous sections). CBH has also been suggested as a cause of late allergic reactions, but has had little experimental support. The "late reaction" may be more relevant to clinical allergy than pure CBH, as it does not seem to be a transient response.

Basophils involved in "late reactions" are different from those in CBH (125). Basophils from CBH infiltrates could not form rosettes with antigens, but one-third of basophils in "late reactions" were rosette-

formers. This may reflect an immunological difference between these two groups of basophils.

Early studies (52,116) demonstrated no evidence of basophilic histamine release. However, Askenase et al. (126) recently described cutaneous basophil anaphylaxis, resulting from tertiary challenge of CBH reactions. Vasoactive amines were released from basophils, and the number of basophils decreased 50% within 20 minutes of challenge. This evidence confirms a functional role for basophils in CBH reactions -- a depot for further immunological attack, directed to the "battlefield" by the lymphocyte.

In addition to mediating local immune responses, basophils have been involved in systemic immunity, systemic cutaneous basophil hypersensitivity (127). This response can best be elicited at 5-7 days after sensitization with soluble proteins or sheep erythrocytes. The response consists of a generalized maculopapular rash, maximal at 18-24 hours after challenge. Microscopically, the rash resembles CBH reactions. The rash is accompanied by eosinophilia, and subsequently basophilia at 48 hours. The significance of this response is not clear.

It is obvious that there is much confusion concerning the clinical significance and mediation of late allergic responses. This study documents further attempts to elicit late cutaneous and nasal responses, with special emphasis on the role of eosinophils and basophils -- analyzing both blood and nasal samples.

MATERIALS AND METHODS

Overall Design

Volunteers with allergic rhinitis participated in preliminary testing, for the purpose of selecting those individuals most likely to produce a LNR, via NPT. Preliminary testing consisted of: 1) standardized low-dose battery of intradermal skin tests; 2) repeat high-dose testing. All subjects participated in the former, to select subjects with a marked immediate cutaneous response. Some subjects also participated in the latter, to select subjects with a LCR; more likely following higher doses of antigen (14,46). Late allergic responses are more likely in highly sensitive individuals (14), and elicitation of the LNR has been correlated with the LCR (24,26).

Preliminary Skin Testing

Volunteer subjects with allergic rhinitis were recruited during the period October 1979 to January 1980. All subjects signed and received a copy of a consent form approved by the Human Investigation Committee of Yale University School of Medicine and Yale-New Haven Hospital. The individuals skin tested ranged in age from 22 to 55. Previous immunotherapy was not a determinant for subject eligibility, but individuals on chronic antihistamine, DSCG, or steroid therapy were excluded.

All individuals received a standardized battery of 11 intradermal skin tests. No antihistamines were taken in the 24 hours preceding testing. The allergen extract was injected through a #27 gauge needle on the posterior surface of the upper arm (5-6 tests on each arm). A minimal amount of allergen sufficient to produce a wheal (minimum-wheal)

was used for each test (approximately .02 ml). The skin tests were read at 15 minutes after injection.

Allergens. All allergens were made from stock solutions of 10,000 or 20,000 protein nitrogen units (PNU)/ml, obtained from Center Laboratories. Dilutions were made serially in sterile 0.4% phenol-0.9% saline. (This investigator used standard solutions for the Allergy Clinic of Yale-New Haven Hospital.)

The following allergen extracts were used:

1) dust 1000 PNU/ml (house dust)
2) dog 100 PNU/ml (dog epithelium)
3) cat 100 PNU/ml (cat epithelium)
4) grass 100 PNU/ml (mixed grasses--made from timothy, orchard, June, red top, and sweet vernal pollens)
5) ragweed 100 PNU/ml (mixed ragweed--made from tall and short ragweed pollens)
6) plantain 100 PNU/ml (English plantain pollen)
7) Alternaria 100 PNU/ml (a mold)
8) horse 100 PNU/ml (horse epithelium)
9) feathers 100 PNU/ml (mixed feathers--chicken, duck and goose)
10) trees 100 PNU/ml (mixed trees--ash, beech, birch, elm, hickory, maple, oak, and poplar pollens)
11) Hormodendrum 100 PNU/ml (a mold)

Grading of skin tests. Skin tests were graded on the following semi-quantitative scale:

0 no visible or palpable wheal

1+ wheal less than 10 mm in diameter, surrounded by erythema

2+ wheal 10-15 mm in diameter with erythema

3+ wheal greater than 15 mm in diameter with erythema

4+ wheal greater than 15 mm in diameter with erythema, and with pseudopods

Repeat (High-Dose) Skin Testing

Subjects received 2-3 intradermal skin tests. All allergens used elicited a 2+ or greater response in preliminary testing. Dose was usually 10-fold greater than in preliminary test, although the low dose was repeated for some subjects. One test was placed on each arm, using the method described above. (In addition, one subject received a third test on the volar surface of the forearm.)

Skin tests were read at 15 minutes, 6 hours, and 24 hours after inoculation. The diameter, in two perpendicular directions, of the lesion (wheal at 15 minutes, induration at 6 hours and 24 hours) was recorded.

Nasal Provocation Testing

Selection of subjects. Subjects were selected on the basis of preliminary test results. Criteria for selection were as follows: 1) No significant history of bronchospasm. 2) A result of 3+ or 4+ on preliminary testing to an allergen, with a corroborative clinical history. 3) No significant sensitivity (0 or 1+) to allergens encountered during a routine day (i.e. pets, dust, occupational exposure). 4) No current rhinitic symptoms off all anti-allergy medications.

Nasal provocation. All nasal provocation was performed during the period December 1979 to January 1980. Testing was begun between 8 A.M. and 10 A.M. Prior to testing, blood and nasal samples were obtained, and baseline nasal resistance was determined.

A total of 0.4 ml (82) of allergen (10,000-20,000 PNU/ml) was alternately sprayed into both nostrils, with forced sniffing. A plastic atomizer bottle, capable of delivering its entire volume, was used for this purpose.

Nasal sections were collected at 10-15 minutes after provocation. Blood samples and nasal resistance measurements were also obtained at 15 minutes after provocation.

Blood and nasal samples, and nasal resistance measurements were repeated at 7 hours and 24 hours after provocation.

Nasal secretions. Subjects were asked to blow (or sneeze) into small plastic (Glad) bags. A small sample from each bag was smeared onto glass slides using a wooden applicator.

Blood samples. Whole blood was collected in purple Vacutainer (Becton-Dickinson) tubes (0.1 ml EDTA additive). Additional samples (serum) were obtained for future analysis.

Rhinomanometry. The measurement of nasal resistance utilized the technique of rhinomanometry (RM). Measurements were made on a Cottle Nasal Pressure/Flow Recorder -- Model PF-102 (Instrumentation and Control Systems, Inc.). (See Figure 1).

FIGURE 1

Rhinomanometer (Cottle Nasal Pressure/Flow Recorder)
Note flow meter in upper left corner.

An anterior rhinomanometric method (74) was used for this study. Two tubes were connected to the subject's nose, one in each nostril (see Figure 2). Air flowed through one tube, during inspiration, while the other tube was connected to the pressure transducer. Separate measurements of each nostril, reversing tubes, were obtained at several flow rates (1-6 L/min). The subject was asked to maintain a constant flow, utilizing the flow meter mounted on the apparatus (see Figure 1). Simultaneous pressure recordings were obtained on the strip recorder (see Figure 3).

Nasal resistance was calculated by dividing the recorded pressure by the known flow -- using an assumption of linearity at low flow rates (29,77,78) -- and averaging the resistance at several flow rates (see Figure 3).

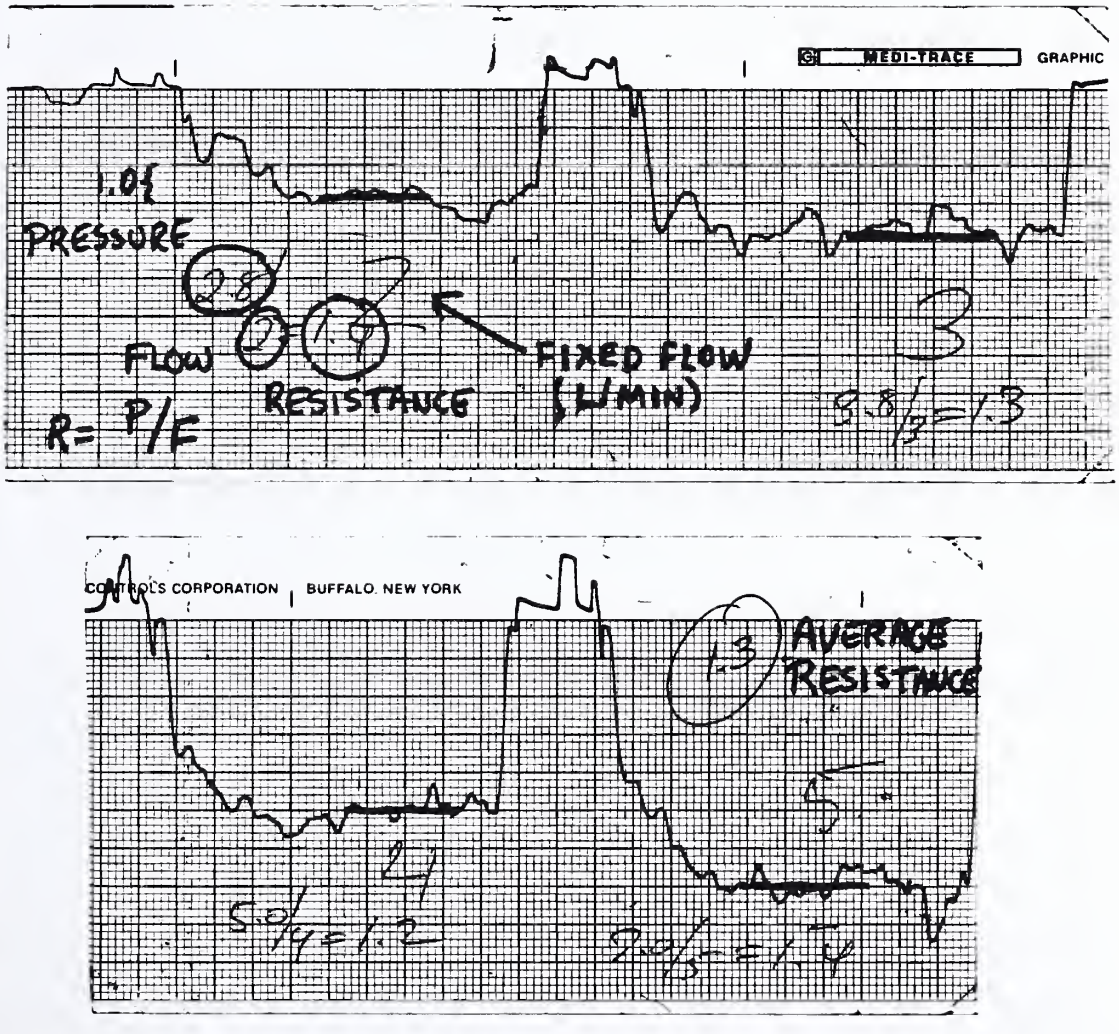
Total nasal resistance. The total resistance (Rt) of the nose is equivalent to the sum of the two parallel "resistors", the right (Rr) and left (Rl) nostrils. Therefore, $1/Rt = 1/Rr + 1/Rl$ (75), or $Rt = (Rr \times Rl)/(Rr + Rl)$.

Nasal ratio. The nasal ratio (NR) is defined as the ratio of the resistance of the "more obstructed" to the "less obstructed" nostril. The definition of "more obstructed" is determined at the first post-provocation measurement (15 minutes), and does not change with future measurements and therefore can be less than 1.0.

FIGURE 2

Subject using rhinomanometer, one tube in each nostril.

FIGURE 3



Pressure tracing for one subject (MZ) (left nostril, 24 hours post-provocation) at several flow rates.

The subject was first instructed to maintain a nasal flow of 2L/min (top tracing, left). The recorded pressure was 2.8, with a calculated resistance of 1.4. The calculated resistances at 3, 4 and 5 L/min were 1.3, 1.2, and 1.4, respectively; yielding an average resistance of 1.3.

Nasal Smears

Stains. Slides were immediately fixed in ethanol for at least 30 minutes before staining with Giemsa or toluidine blue. Giemsa stain (original azure blend type, Harleco), 7.415 g/l in methanol, was diluted 1:20 in distilled water immediately prior to use. 1% toluidine blue was obtained from the Clinical Hematology Laboratory, Yale-New Haven Hospital, and diluted 1:20 in ethanol before use.

Slides were stained in Giemsa for 15-30 minutes, and then rinsed gently in tap water.

For comparison, identical slides were stained for 5 minutes in 0.05% toluidine blue, and then rinsed by 5 dips in ethanol, and air dried (53).

Nasal cytology. Each slide was assessed semi-quantitatively for total cells, epithelial cells, neutrophils, lymphocytes, eosinophils, and basophils. The method used is as follows:

0 no cells seen on entire slide

1+ at least one cell seen

2+ two or more cells in same low-power field (LPF) (100X)

3+ three or more cells in same LPF, and two or more cells in same high-power field (HPF) (970X), within that LPF

4+ two or more HPFs containing two or more cells, within one LPF

5+ two or more 4+ LPFs on same slide

In addition, the amount of debris was defined as the total cellularity (0-5+) minus the maximum grade for any individual cell.

Leukocyte Counts

Total white blood cell count (WBC). The tube was gently mixed immediately prior to dilution, to prevent separation of cells and plasma. 0.025 ml of blood was diluted in .475 ml 3% glacial acetic acid, using a Unopette test 5856 (Becton-Dickinson). The mixture was gently shaken to ensure proper mixing, and allowed to sit at room temperature for 10-30 minutes, ample time for erythrocyte hemolysis. 0.009 ml of blood-acetic acid solution was added to each chamber of an Improved Neubauer hemocytometer. Both chambers (0.1 mm^3 each) were counted, and the total number of leukocytes multiplied by 100 (20-fold dilution, 0.2 mm^3 counted). If there was a significant difference between the chambers, a repeat count was made.

Differential. A drop of blood was placed on a glass slide, gently smeared using a cover slip, and allowed to dry. Slides were stained with Wright's stain (obtained from Clinical Hematology Laboratory) for 3 minutes, followed by the addition of Wright's buffer (obtained from same source) for 7 minutes. Slides were then rinsed in tap water and allowed to air dry.

100 leukocytes were counted under high power (970X). Segmented neutrophil (seg), band neutrophil (band), lymphocyte, monocyte, eosinophil and basophil counts were determined by multiplying the percentage of each by the WBC.

Statistical Methods

Nonparametric methods were used for all analyses of significance (128); including the Wilcoxon signed rank test, the Wilcoxon rank sum test, and Spearman's rank correlation.

RESULTS

Preliminary Skin Testing

23 individuals participated in preliminary skin testing. 16/23 (70%) were highly sensitive (3+ or 4+) to at least one of the allergens used. The sensitivity profile of the atopic subjects is shown in Table III. Dust, grass, and ragweed were the most common sensitivities in this group.

Elicitation of LCR

6 subjects received high-dose skin testing (total of 13 tests) in order to assess the frequency of the LCR. The results are seen in Table IV. A significant LCR persisting at least 24 hours could be elicited in all subjects, to at least one allergen.

One subject (JGa) had taken an antihistamine several hours prior to testing. There was a marked decrease in the immediate response (compared to previous testing), but it was followed by a significant LCR.

There was a general trend towards larger LCRs following larger immediate responses; but the correlation was not statistically significant.

Nasal Provocation Testing

Immediate nasal response. All 11 subjects had at least a mild clinical response (see Table V), with most (9/11) demonstrating significant symptoms of sneezing, rhinorrhea, and obstruction, within 15 minutes following NPT.

TABLE III

Allergic Profile of Subjects

Subject	Allergens 1	2	3	4	5	6	7	8	9	10	11
A. Subject of nasal provocation testing											
DB	0	0	0	4+	3+	0	0	0	0	0	0
MZ	3+	0	0	0	0	0	0	0	0	0	0
JGi	3+	3+	2+	3+	3+	0	1+	1+	0	0	1+
JGa	3+	3+	0	3+	1+	0	1+	0	0	0	0
RD	0	1+	0	4+	0	0	0	0	0	0	0
SD	1+	3+	1+	4+	1+	0	1+	0	0	0	1+
RZ	3+	0	2+	2+	4+	1+	0	1+	0	0	0
LS	3+	1+	0	0	3+	1+	1+	0	0	1+	0
BM	3+	3+	3+	4+	1+	4+	2+	1	0	0	0
JN	3+	0	3+	0	3+	0	3+	0	0	0	0
PG	1+	0	0	4+	4+	2+	1+	0	0	4+	0
B. Other highly sensitive subjects (at least one 3+ or 4+ response)											
DO	2+	1+	4+	3+	0	4+	0	1+	0	4+	0
GC	0	0	3+	0	0	0	0	0	0	0	0
MR	2+	0	0	0	3+	0	3+	0	0	0	3+
AE	3+	2+	4+	0	1+	1+	0	2+	1+	1+	2+
RL	3+	3+	2+	4+	0	0	0	4+	1+	0	0
C. % of subjects with given sensitivity											
Sensitivity											
3+ or greater	56%	31%	31%	56%	44%	12%	12%	6%	0%	12%	6%
2+ or greater	69%	38%	50%	62%	44%	19%	19%	12%	0%	12%	12%

Key to allergens:

1) dust
2) dog
3) cat
4) grass
5) ragweed
6) plantain
7) Alternaria
8) horse
9) feathers
10) trees
11) Hormodendrum

TABLE IV

The Relationship of the Immediate to the Late Cutaneous Response

Subject	Allergen	Size of response (mean diameter in mm) Time after injection 15 min	6 hr	24 hr	Low-Dose Response
MR	Alt. 1000	9 x 11 (10)	16 x 16 (16)	40 x 40 (40)	(3+)
	Tree 1000	12 x 13 (12.5)	70 x 70 (70)	60 x 50 (55)	(0)
RZ	Dust 1000	7 x 8 (7.5)	14 x 14 (14)	(0)	(3+)
	Rag. 100	30 x 10 (20)	50 x 50 (50)	55 x 75 (65)	(4+)
	Grass 1000	11 x 10 (10.5)	40 x 40 (40)	(0)	(2+)
AE	Dust 10,000	10 x 7 (8.5)	30 x 50 (40)	25 x 25 (25)	(3+)
	Cat 1000	12 x 12 (12)	35 x 35 (35)	32 x 27 (29.5)	(4+)
JGa*	Dust 10,000	8 x 5 (6.5)	72 x 75 (73.5)	60 x 50 (55)	(3+)
	Grass 1000	8 x 4 (6)	65 x 70 (67.5)	40 x 40 (40)	(3+)
JGi	Rag. 1000	20 x 13 (16.5)	33 x 36 (34.5)	30 x 35 (32.5)	(3+)
	Grass 1000	15 x 11 (13)	33 x 25 (29)	34 x 40 (37)	(3+)
DB	Rag. 1000	8 x 7 (7.5)	30 x 38 (34)	45 x 50 (47.5)	(3+)
	Grass 1000	24 x 18 (21)	70 x 75 (72.5)	75 x 75 (75)	(4+)

*Drixoral taken several hours prior to testing

TABLE V

Nasal Provocation Testing

Subject		Rr	Rl	Rt	NR	Clinical Response
DB	A	1.3	1.1	0.6	0.8	
	B	1.5	2.0	0.9	1.3	marked sneezing, obstruction
	C	1.3	1.2	0.6	0.9	none
	D	1.5	1.2	0.7	0.8	none
MZ	A	1.0	1.1	0.5	0.9	
	B	1.5	1.0	0.6	1.5	mild coryza, obstruction
	C	1.0	1.0	0.5	1.0	none
	D	0.8	1.3	0.5	0.6	none
JGi	A	2.0	1.0	0.7	2.0	
	B	+	-	+	+	marked sneezing, obstruction
	C	6.0	0.3	0.3	20.0	none
	D	5.9	0.6	0.5	9.8	none
JGa	A	1.2	1.1	0.6	0.9	
	B	1.2	1.5	0.7	1.1	marked sneezing
	C	1.3	1.4	0.7	1.1	"packed sinuses"
	D	1.2	1.3	0.6	1.1	"congested in back"
RD	A	1.2	1.3	0.6	0.9	
	B	2.6	1.7	1.0	1.5	marked sneezing, obstruction
	C	1.2	1.2	0.6	1.0	none
	D	1.1	1.3	0.6	0.8	none
SD	A	1.2	1.2	0.6	1.0	
	B	+	-	+	+	marked sneezing, obstruction
	C	1.1	1.3	0.6	0.8	headache
	D	1.2	1.3	0.6	0.9	none
RZ	A	1.4	1.4	0.7	1.0	
	B	1.3	1.4	0.7	1.1	mild coryza
	C	1.2	1.3	0.6	1.1	none
	D	1.1	1.1	0.6	1.0	none
LS	A	1.1	1.3	0.6	1.2	
	B	-	+	+	+	marked sneezing, obstruction
	C	1.0	1.9	0.7	1.9	none
	D	1.6	0.9	0.6	0.6	none

TABLE V (continued)

Nasal Provocation Testing

Subject		Rr	Rl	Rt	NR	Clinical Response
BM	A	1.2	1.1	0.6	1.1	
	B	6.4	0.4	0.4	16.0	marked sneezing, obstruction angioedema of uvula and palate
	C	1.2	1.2	0.6	1.0	moderate angioedema
	D	1.2	1.1	0.6	1.1	none
JN	A	1.1	1.2	0.6	0.9	
	B	2.0	1.2	0.8	1.6	moderate sneezing, obstruction
	C	1.5	1.2	0.7	1.2	none
	D	1.1	1.1	0.6	1.0	sore throat
PG	A	5.7	1.0	0.9	0.2	"not congested"
	B	1.1	6.1	0.9	5.5	marked sneezing, obstruction
	C	4.9	0.8	0.7	0.2	none
	D	3.2	1.2	0.9	0.4	none

A	pre-provocation	+	unmeasurably high
B	15 min. post-provocation	-	unmeasurably low
C	6 hr. post-provocation		
D	24 hr. post-provocation		

The rhinomanometric data was somewhat confusing. Three subjects (JGi,SD,LS) were virtually totally obstructed at 15 minutes, as the nasal resistance was unmeasurably high on one side. Simultaneously, the resistance on the other side was close to zero, as determined by RM. Total resistance in this case was judged "+" (infinitely high, for statistical purposes).

One subject (BM) had a marked increase in Rr, with a concomittant decrease in Rl. However, the calculated Rt was less than pre-provocation.

Overall, there was not a statistically significant correlation between the clinical response, and post-provocation increases in Rt or NR.

Late nasal response. No subjects complained of obstruction at 7 hours or 24 hours post-provocation. Two subjects (DB,BM) reported some mild obstruction in the evening following provocation. One subject (SD) complained of a headache at 7 hours post-provocation, and another (JGa) noted some sinus congestion at 7 hours and 24 hours. In addition, one subject (JN) had a sore throat at 24 hours, of doubtful relation to NPT.

7/11 subjects had no increase in Rt at 7 hours or 24 hours post-provocation (compared to pre-provocation), and the increase was only 0.1 in the other 4 subjects. Only two subjects (JGi,LS) had greater than a 50% increase in NR at 7 hours. JGi had a 900% increase in NR at 7 hours, and a 390% increase at 24 hours. Neither of these subjects noted any clinical symptoms after cessation of the immediate response.

Complication. An unforeseen, and previously unreported complication of NPT was noted in one subject (BM). Severe pharyngeal irritation was noted within 5 minutes after provocation, which progressed to localized

angioedema of the uvula and soft palate within 15 minutes (see Figure 4). There was no dyspnea, or wheezes on physical exam, but phonation was markedly impaired. The subject was offered epinephrine, but refused, preferring to continue with the study. The edema subsided gradually over the next 24 hours.

Leukocyte Counts (Table VI)

The mean WBC and differential counts were within normal limits in each of the four analysis groups (129).

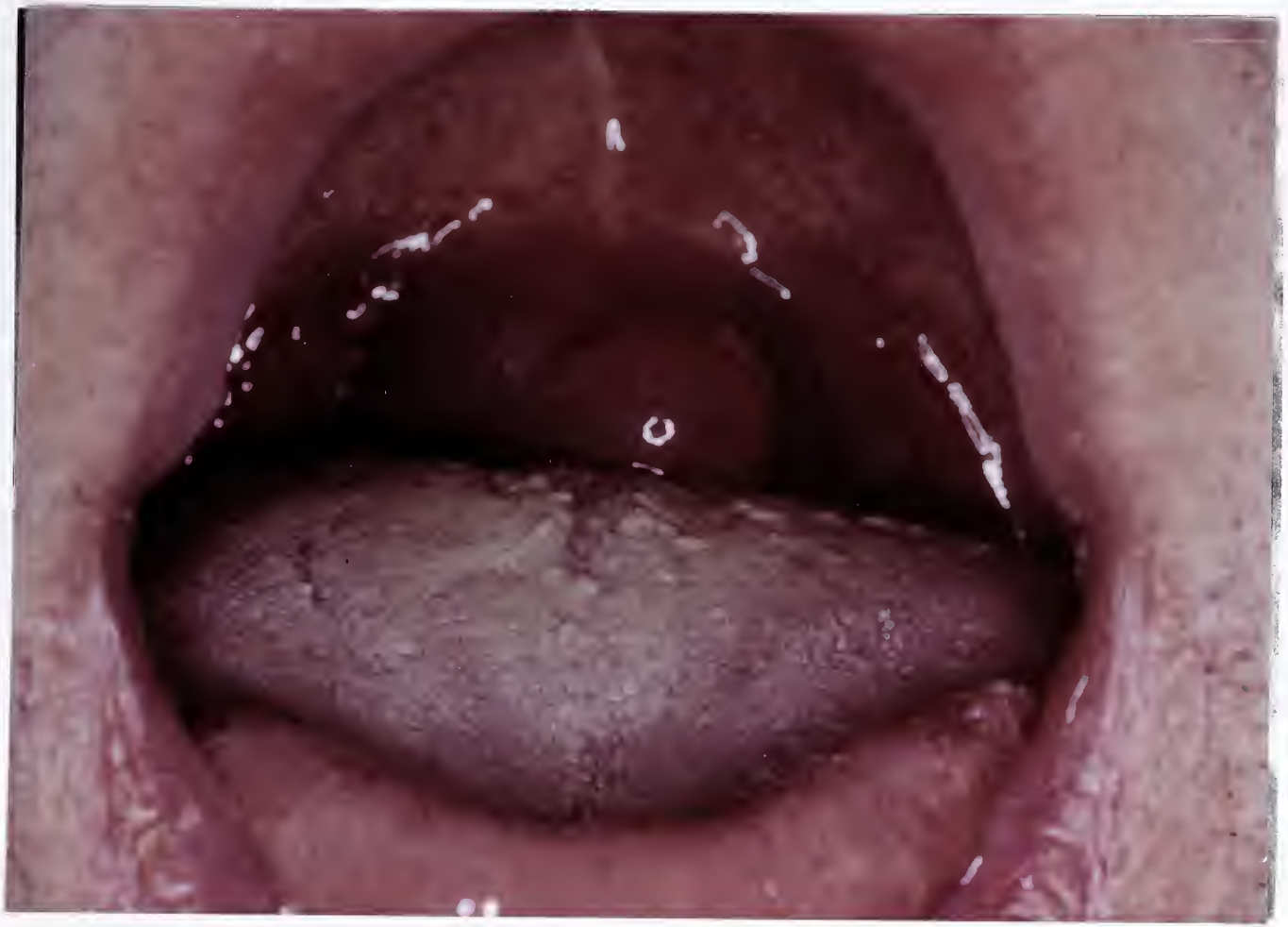
WBC. There was no overall increase in the WBC in the first 15 minutes, as seen in Table VI. However, there was a significant ($p<.05$) correlation between the increase in NR (at 15 minutes) with changes in WBC over the same period (see Table VII).

There was significant ($p<.05$) increase in the WBC at 7 hours vs. pre-provocation, and vs. 15 minutes; including a significant ($p<.05$) increase in segmented neutrophils and monocytes (vs. 15 minutes). Other cell counts (bands, lymphocytes) were also increased, but not significantly ($p>.05$).

Eosinophils (Table VIII). The percentage of eosinophils fell over the first 7 hours post-provocation ($p<.05$) from $2.1 \pm 1.9\%$ (mean $\pm$ S.D.) to $0.9 \pm 1.1\%$. The absolute number of eosinophils also decreased, but not significantly ($p>.05$).

Basophils. Basophils were only seen in the peripheral smears of two subjects (PG,BM).

FIGURE 4



Complication of NPT in one subject (BM) — angioedema of the uvula and soft palate (taken approximately one hour after provocation).

TABLE VI

Leukocyte Counts

Time	WBC	Mean/mm^3 ± S.D. Segs	Bands	Lymphocytes	Monocytes	Eosinophils	Basophils
Pre.	5800 ± 1790	3380 ± 1470	205 ± 170	1820 ± 690	269 ± 273	125 ± 131	0 ± 0
15 min.	5900 ± 1560	3550 ± 1330	245 ± 235	1830 ± 670	221 ± 180	60 ± 81	0 ± 0
7 hr.	7470 ± 2600 *,#	4570 ± 1940 #	283 ± 305	2170 ± 780	369 ± 242 #	78 ± 114	5 ± 17
24 hr	6160 ± 1620	3560 ± 810	188 ± 152	2000 ± 930	250 ± 179	155 ± 147	6 ± 19

* significant ($p<.05$) vs. Pre.

significant ($p<.05$) vs. 15 min.

TABLE VII

Changes in WBC and NR during NPT

	WBC/mm^3			NR		
Subject	Pre	15'	Change	Pre	15'	Change*
DB	4800	3800	-1000	0.8	1.3	0.5
MZ	3600	3700	+ 100	0.9	1.5	0.6
JGi	5900	6700	+ 800	2.0	+	+
JGa	8000	6800	-1200	0.9	1.2	0.3
RD	5700	7000	+1300	0.9	1.5	0.6
SD	4600	4800	+ 200	1.0	+	+
RZ	6800	6900	+ 100	1.0	1.1	0.1
LS	4000	5600	+1600	1.2	+	+
BM	6400	6600	+ 200	1.1	16.0	14.9
JN	9500	8600	- 900	0.9	1.6	0.7
PG	4500	4400	- 100	0.2	5.5	5.3

+ unmeasurably high

* increase in ratio of resistances of individual nostrils

TABLE VIII

Changes in Eosinophils during NPT

	Eosinophils	
Time	Total	%
Pre-prov.	125 ± 131	2.1 ± 1.9
15 min.	60 ± 81	1.2 ± 1.3
7 hr.	78 ± 114	0.9 ± 1.1*
24 hr.	155 ± 147	2.4 ± 2.1

* significant ($p<.05$) vs. Pre-prov.

Nasal Smears

Giemsa. There was no obvious overall difference between the pre-provocation and post-provocation smears. The epithelial cell was most frequently seen (Table IX), followed by neutrophils, with occasional lymphocytes. Eosinophils were only seen in one smear (RZ at 7 hours), and no basophils were visible in this preparation.

There was a significant ($p<.05$) difference (using chi-square) in the frequency of neutrophils among the following two groups of slides: those with 1) many epithelial cells; and 2) none or few epithelial cells (see Table X). The converse was also true (i.e. relating frequency of epithelial cells to frequency of neutrophils).

There was no difference in the frequency of lymphocytes in either set of groups noted above (see Table X).

More succinctly, neutrophils occurred more frequently in the presence of epithelial cells, but lymphocytes appeared independently of both.

Examination of the smears was made difficult by the high frequency of dead, poorly-staining cells, which were commonly fragmented, and difficult to identify. These cells were counted in "total cells", but not individually classified.

Toluidine blue. Very few cells were seen using this staining method, and no overall analysis was attempted.

TABLE IX

Nasal Smears

	% of slides with given frequency of cells (n=44)		
Cell	None	Few	Many
Total cells	4.5%	34.1%	61.4%
Epithelial cells	15.9%	38.6%	45.5%
Neutrophil	65.9%	15.9%	18.2%
Lymphocyte	86.4%	11.4%	2.3%
Eosinophil	97.7%	2.3%	--
(Debris)	56.8%	43.2%	--

TABLE X

Interrelationship Between the Frequency of Occurrence of Epithelial Cells, Neutrophils and Lymphocytes

# of slides with given frequency of specific cell						
	Neutrophil*			Lymphocyte		
Slides with	None (0)	Few (1+-3+)	Many (4+,5+)	None	Few	Many
Many epithelial cells	9	4	7	17	2	1
None or few epithelial cells	20	3	1	21	3	0
	Epithelial Cell*			Lymphocyte		
	None	Few	Many	None	Few	Many
Many neutrophils	0	1	7	6	1	1
None or few neutrophils	7	16	3	32	4	0

*significant ($p<.05$) difference between two groups

DISCUSSION

The Occurrence of the Late Nasal Response

The primary goal of this investigation was to reproducibly elicit the LNR. Some investigators (24,26,29) have noted such a response as a common succession to NPT. Others (28) have relied on a small subset of their subject population for studies on the LNR. Richerson et al. (38) were not able to objectively document the LNR in any of 21 patients tested. The results of this study are in agreement with Richerson's; as there was no definite objective LNR in the 11 subjects examined.

Difficulty of elicitation. It is unclear whether the problem is in elicitation of the LNR, or in diagnosis of such a response. Schumacher et al. (29) were only able to elicit the LNR when nasal threshold doses were exceeded. However, these doses frequently induced severe symptoms of persistent sneezing, palatal and pharyngeal itching, conjunctivitis and headache; and dyspnea was noted in one patient requiring bronchodilators. There is also a danger of systemic anaphylaxis, as a significant quantity of allergen is absorbed through the nasal mucous membrane (87).

A severe reaction was induced in one subject (BM) in this study, angioedema of the uvula and soft palate; affecting phonation, but not respiration. It is not clear whether this was due to the dose of allergen (4000 PNU), or an uneven deposition of allergen to the affected areas.

Elicitation of the LNR may require a more gradual administration of the allergen, as profuse sneezing will expel most of the contents of the nasal lumen. Slavin et al. (25) used nasal packs for provocation, which resulted in a non-immediate response -- not fully characterized.

Another possibility is a requirement for priming, prior to the administration of allergen. Connell's studies (88-91) on the "priming effect" note anatomical changes in the nasal mucosa after repeated challenge. It is possible that similar changes are necessary for the elicitation of the LNR. Some individuals might have permanent changes, and would comprise a small subset which could readily produce a LNR.

Difficulty of observation. The LNR may be a common response, but difficult to diagnose by some techniques. In those patients with a marked response, diagnosis would be obvious. But some subjects, as in this study, complain of mild obstruction or other late symptoms, such as sinus congestion or headache. Rhinomanometry failed to confirm a LNR in this study.

Clinical late responses may be due to posterior obstruction, which may not significantly change nasal resistance. This is analogous to the proposed mechanism of mucosal edema of the small airways, in the LBR (17), which may not decrease peak expiratory flow.

Total nasal resistance (Rt) was not a reliable indicator of nasal obstruction, as one subject's (BM) Rt decreased following NPT, simultaneous with a marked clinical response. This was probably due to artifactually low resistance readings in the less obstructed nostril, possibly a result of poor pressure transmission through the more obstructed side. In addition, uneven deposition of allergen could have resulted in congestion of one nostril, with reciprocal decongestion of the opposite nostril (130), causing uncertain effects on Rt.

Rapid changes in the NR may be useful as a guide to an immediate response, but changes occurring over several hours may simply be a manifestation

of a normal physiologic nasal cycle (75,131,132). Heetderks (131) first demonstrated the existence of a nasal cycle, occurring over lengths of time ranging from 30 minutes to 4 hours. Hasegawa and Kern (75) found at least one complete cycle in 72% of normal subjects, with a mean duration of 2.6 hours. Cycles varied from 1 to 6 hours, longest in older subjects.

If there is deviation of the nasal septum, there will be a high baseline NR. This could also obscure the existence of a LNR, a possibility in one subject (PG) in this study.

In addition, it is difficult to control for the effects of position or temperature changes (133), and directed light or air flow (134).

It may be best to rely on clinical diagnosis. Malmberg et al. (135) found RM less sensitive than clinical criteria in detecting a positive nasal response. The results of this study also suggest that RM is not a reliable method of assessing nasal reaction, although diagnosis could probably be markedly improved with more elaborate equipment.

Changes in Leukocytes Following Nasal Provocation

Leukocytosis is sometimes seen in late bronchial responses (5,16,24). Therefore, it was considered that changes in leukocytes might occur in association with the late nasal response. However, as noted above, there was no clear documentation of the occurrence of the LNR in this study.

Changes in WBC. The WBC increased over the 7 hours following provocation, from mid-morning to late afternoon. It is unlikely that this is due to an immune response, as the WBC normally is higher in the afternoon, peaking at around midnight (136,137). The diurnal rhythm is probably a result of changes in corticosteroid levels (138). In addition,

changes in light, exercise, or stress can all affect the WBC (137). As this study did not use a non-provoked control group, no conclusions can be drawn concerning the effect of NPT upon the WBC.

Also noted was a correlation between WBC changes, and increases in the NR, at 15 minutes post-provocation. This is most likely related to the stress of provocation, greater in those with a more severe immediate response.

Changes in segmented neutrophils and monocytes. Changes in neutrophil counts tend to be parallel to those of the WBC (137). The increase in monocytes at 7 hours (vs. 15 minutes) was unexpected, and its etiology is not clear.

Changes in eosinophils. The normal eosinophil count decreases during the morning hours, with a nadir around noon (136,137,139-144). The eosinophil count rises during the early afternoon, peaking around midnight. Late afternoon counts are usually slightly higher than morning values.

In this study, there was an insignificant decrease in eosinophils following provocation, but a significant decrease in the percentage of eosinophils. This result is interesting, as one would expect a control group to have slightly higher eosinophil counts at 7 hours post-provocation (late afternoon). In addition, following bronchial provocation, eosinophil counts increased at 6 hours (24); although this was followed at 24 hours by a late response.

Unfortunately, absolute eosinophil counts were not done, and further studies are required before concluding the existence of eosinophil changes following nasal provocation.

Occurrence of the Late Cutaneous Response

The frequency of the LCR was 100% in the 6 subjects tested. This required high doses of allergen (approximately 200 PNU). Robertson et al. (14) have demonstrated similar results. As also described in prior studies (38), larger immediate responses tended to yield larger LCRs.

Changes in Nasal Cytology Following Nasal Provocation

It was considered that changes in the eosinophil or basophil population of the nasal mucosa might occur at the time of the LNR. However, there was no obvious change in any component of the nasal smear, at any time following NPT.

Eosinophils were a rare entity, probably requiring more than one provocation to be noted in the nasal smear.

Basophils have previously been found in the nasal secretions of patients with allergic rhinitis, but only when symptomatic (53). Again, one provocation is probably not a significant enough challenge to adequately induce a model of allergic rhinitis.

It is possible that basophils and/or eosinophils were present, but destroyed in the preparation of the slides. Further refinement of this technique may yield more conclusive results regarding the population of the provoked nasal mucosa.

The liberation of neutrophils and epithelial cells into nasal secretions may have a common basis, as the occurrence of many epithelial cells was often associated with a high frequency of neutrophils. However, lymphocytes occurred independently, suggesting that there is little interaction between lymphocytes, and neutrophils and/or epithelial cells.

SUMMARY

1) The LNR is difficult to elicit and/or document.

2) In contrast, the LCR can be elicited in virtually all atopic subjects, using suprathreshold intradermal doses.

3) Nasal provocation testing may be useful clinically as a test for sensitivity, but a single provocation does not yield a complete experimental model for allergic rhinitis.

4) Attempts to induce late allergic responses using high doses of allergen may be extremely dangerous.

5) The clinical significance and mediation of the LNR is still unclear.

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