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Allergen-Specific Nasal Challenge: Response Kinetics of Clinical and Inflammatory Events to Rechallenge

Key Words

Allergen-specific nasal challenge
Rechallenge
Inflammation

Abstract

Allergen-specific nasal challenge is a valid and reliable tool for studying the pathophysiological mechanisms involved in allergic inflammation. Nasal challenge induces an immediate clinical response in allergic subjects and a concomitant appearance of an inflammatory infiltrate. The mucosal inflammation may persist up to 48–72 h after allergen exposure. If the subjects are rechallenged within this period the response is more pronounced: the so-defined priming effect. The aim of the study was to evaluate the effects of the nasal rechallenge, performed at different time intervals: 3 days, 1, 2 and 4 weeks after the first challenge. Forty allergic subjects underwent two nasal challenges: at baseline and after the periods mentioned above (10/group). Symptoms and inflammatory cells (eosinophils and neutrophils recovered by nasal brushing) were assessed. The 3-day-interval group showed a hyperreactive response (priming effect), the 1- and 4-week-interval groups showed a response similar to baseline, and the 2-week-interval group showed a hyporeactive response ('tolerogenic effect'). The last phenomenon may be due to a possible immunologic response similar to that achievable during local specific immunotherapy. The present results further elucidate the kinetics of allergen-driven inflammatory events and highlight the importance of the time period chosen for rechallenge. The latter fact may be of primary importance in clinical trials involving specific challenge.

Introduction

Allergic rhinitis is the most common immunologically mediated disease and its prevalence is continuously increasing. It is characterized by typical clinical events: sneezing, nasal itching, rhinorrhea and congestion. All these symptoms are caused by mediators released from different inflammatory cells. In fact, the nasal mucosa is constantly infiltrated by inflammatory cells (mainly eosinophils, neutrophils and metachromatic cells) during the allergic reaction

[1, 2]. These cells are also present in the nasal mucosa in their activated form, releasing mediators, chemotactic factors and cytokines, which all play a role in the maintenance of allergic inflammation [1].

Allergen-specific nasal challenge (ASNC) is a suitable method for studying the pathophysiological mechanisms, which sustain the allergic inflammation, since it is a safe, reproducible, and reliable test [3, 4]. Nasal challenge induces an immediate clinical response in sensitized subjects, very similar to that occurring after natural exposure to allergen

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(so-called early phase reaction), which lasts for a short time so that it was defined as immediate hypersensitive reaction [4]. A concomitant appearance of an inflammatory infiltrate (neutrophils, eosinophils and metachromatic cells) is also detectable during this phase [3].

Inflammatory cell recruitment is a very complex phenomenon, involving several mechanisms and occurring in different steps: the final event is represented by inflammatory cell infiltration of the nasal mucosa. A main pathogenetic mechanism is the involvement of the adhesion molecule system, which makes possible the interaction between molecules expressed on the surface of inflammatory and epithelial cells [5, 6]. All these events induce and maintain a mucosal inflammation, which may persist up to 48–72 h after a single allergen exposure [3]. This inflammatory status accounts for two main clinical phenomena: (1) if a subject is rechallenged 24–48 h after a challenge with the same allergen-provoking dose, the response (both clinical and inflammatory) is more intense: the so-defined ‘priming effect’ and (2) during natural allergen exposure (e.g. pollen season) the allergic subject becomes more susceptible to subsequent exposures; this is defined as ‘specific hyperreactivity’, which is similar to the nonspecific hyperreactivity, both being related to inflammation [3].

To better define the different steps of allergen responsiveness, we studied the kinetics of these phenomena; thus, we evaluated the effects of nasal rechallenge, performed at different time intervals, both on symptoms and inflammatory cell infiltration, monitoring clinical and cytological parameters.

The fact that in the literature several studies have been reported is not ignored [3, 4, 7–10], but it is difficult to compare them, since different challenge protocols were adopted or different intervals between challenge and rechallenge were used. The results of the present study should be valuable both in reviewing the previous studies and in designing new ones.

Materials and Methods

Study Design

The study was designed as four parallel groups. The aim was the evaluation of clinical and cytological parameters by means of ASNC performed at different time intervals. Grass-sensitive patients with allergic rhinitis were studied outside the pollen season, when they were symptom-free. ASNC was performed according to our previous reports [3]. The subjects were subdivided in four groups: (1) rechallenged after a 3-day interval, (2) after 1 week, (3) after 2 weeks, and (4) after 4 weeks. The study was performed according to the Declaration of Helsinki; informed consent according to the rules of the ethical committee of our department was obtained from each patient.

Subjects

Forty subjects, 23 females and 17 males (aged between 18 and 49 years, mean 32.5), were enrolled in the study. All subjects suffered from seasonal allergic rhinitis caused solely by grass pollens. All of them experienced symptoms at least in the 2 previous years and a positive skin prick test ($>3+$, histamine= $3+$) for the specific allergen was ascertained. All subjects were completely asymptomatic. Local nasal examination was negative in all subjects. On admission, no subject had suffered from any upper or lower airway respiratory disease in the 6 weeks preceding the study. They were not treated with any topical or systemic drug in the preceding 4 weeks and during the study and they had not previously received specific immunotherapy.

Allergen

The grass extract (the same employed for the skin prick test, kindly provided by DHS-Bayer, Milan, Italy), stored as a lyophilized powder at 4°C, was reconstituted in the diluent (saline human albumin, 0.03%) within 24 h before the test. The concentration was expressed as allergen unit by RAST (AUR)/ml; different dilutions were employed: 25, 50, 100, 250 and 500 AUR/ml.

Allergen-Specific Nasal Challenge

Nasal challenge was performed following the methodology previously described by our group [3]. Briefly, two 10-ml lavages with lactated Ringer’s solution at 37°C were performed to establish a stable baseline. A 0.05% solution of oxymetazoline hydrochloride (0.1 ml, Nasivin, Bracco, Italy) was then applied. This drug is an α -adrenergic agonist, which prevents excessive congestion of the nasal mucosa after allergen challenge and allows the continued collection of nasal specimens, guaranteeing nasal patency. In addition, oxymetazoline does not influence mediator release [11].

Challenges with the vehicle used for the allergen (0.03% saline albumin) were performed to rule out any nonspecific hyperreactivity. A series of challenges with increasing doses of allergen (as mentioned) was then begun at standard time intervals (15 min) until a clinical nasal hypersensitivity reaction was elicited. The initial allergen concentration used for nasal challenge was the same as the end point skin prick test. Allergen solutions were sprayed into one nostril using a metered pump delivering 90 ± 2 μ l per puff, with the patient holding his or her breath in full inspiration to avoid bronchial provocation. After spraying, patients were allowed to wipe their nose but not to blow it. Evaluations were performed at baseline and 30 min (early phase reaction) after the clinical reaction, before and after the established interval.

Clinical Evaluation

The clinical reaction was assessed as total symptom score according to an arbitrary four-point rating scale, from 0 to 3 (0 = absent, 1 = mild, 2 = moderate, 3 = severe), increasing for severity in both nostrils, according to our previous reports [3]. Nasal itching, sneezing, rhinorrhea, and congestion were considered. Signs were evaluated separately by two physicians, and symptoms were spontaneously reported by the subjects. A total symptom score, after challenge, exceeding 7 was considered a positive response, according to our previous studies, and the allergen threshold dose was thus defined for each subject. The second challenge (rechallenge) was performed using the same procedure until the threshold dose was achieved.

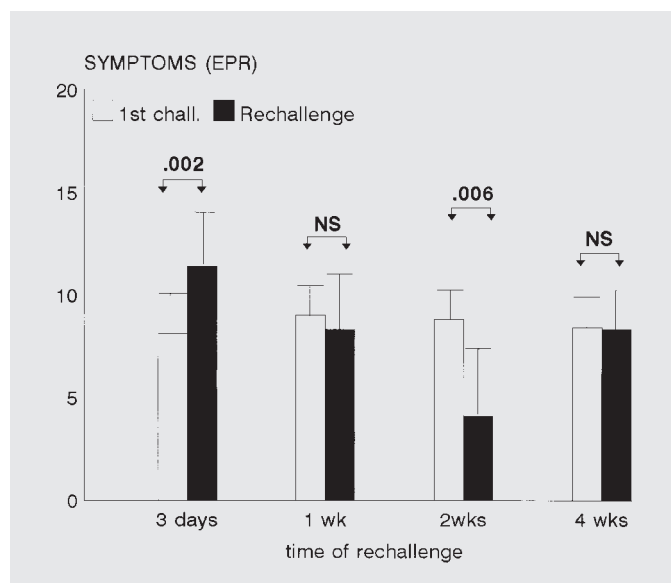


Fig. 1. Specific nasal challenge symptom score (mean + SEM) at the EPR in the four groups of patients (i.e. rechallenged after 3 days, 1, 2 or 4 weeks). A significant increase of response is evident in group 1, whereas a significant decrease is evident in group 3. The intragroup comparison p values are plotted above the bars.

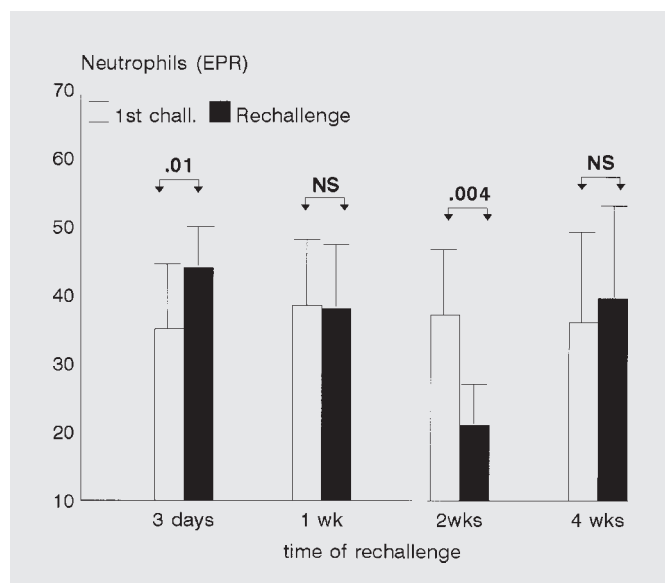


Fig. 2. Neutrophil count in nasal scraping at the EPR of specific challenge (mean + SEM) in the four groups of patients (i.e. rechallenged after 3 days, 1, 2 or 4 weeks). The intragroup comparison p values are plotted above the bars.

Cytological Assessment

Nasal scrapings were obtained and processed as previously described [3]. Briefly, the cotton tip was immersed, after the scraping, into a plastic tray with PBS and transferred to a 10-ml polypropylene tube. The recovered fluids were centrifuged at 1,400 g for 10 minutes and each pellet resuspended in PBS solution (2 ml); cell suspensions were filtered to reduce the quantity of mucus and cytospin slides were prepared using standard techniques. The differentiation among eosinophils, neutrophils and epithelial cells was made by May-Grünwald/Giemsa staining. The slides were read using the optic microscope (Leitz Laborlux Microscope, 250 × focus). The number of inflammatory cells (neutrophils, eosinophils) was considered as a total number of any cell type/every microscope field: the data are expressed as a mean of 10 fields. Slides were examined by two investigators unaware of the identity of the samples.

Statistical Analysis

Wilcoxon and Mann-Whitney nonparametric tests were employed for intragroup and intergroup comparison at different times, respectively.

Results

All patients completed the study and developed an early phase reaction (EPR) following a specific challenge. They were all symptom-free and the baseline scrapings performed

before challenge showed no inflammatory infiltration except for one group, as detailed below.

Clinical Parameters

The group rechallenged 3 days after the first ASNC showed a significantly more prominent clinical response ($p = 0.002$) as reported in figure 1. The group rechallenged after 14 days showed a less intense ($p < 0.01$) clinical response compared to the first challenge. The groups rechallenged after 7 and 28 days showed a response comparable to that observed during the first challenge ($p = \text{non-significant}$). The data are summarized in figure 1.

Cytological Assessment

Consistently with the clinical features, the groups rechallenged after 7 and 28 days showed a neutrophilic (fig. 2) and eosinophilic (fig. 3) infiltration comparable to the first challenge ($p = \text{non-significant}$). The group rechallenged after 14 days showed a less prominent cellular infiltration ($p < 0.05$) at the EPR. Finally, the group rechallenged after 3 days showed a significant cellular infiltration even before challenge ($p = 0.05$ and 0.01 ; fig. 4), and had a more pronounced infiltration at the EPR compared to the first challenge ($p = 0.01$ and 0.02).

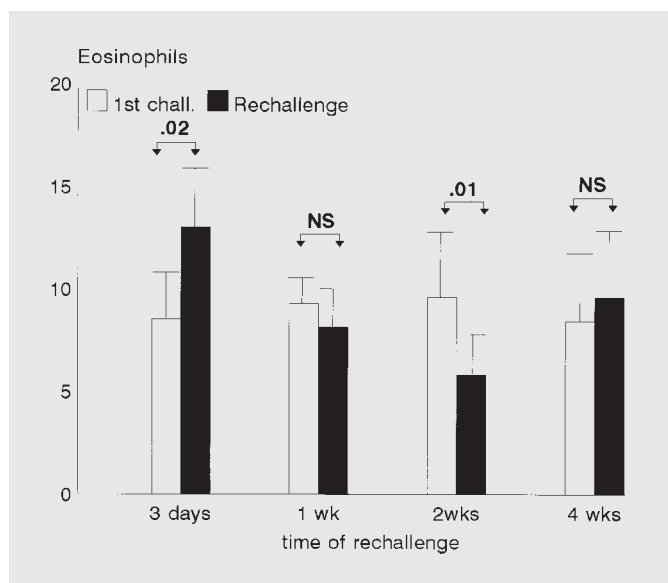


Fig. 3. Eosinophil count in nasal scraping at the EPR of specific challenge (mean + SEM) in the four groups of patients (i.e. rechallenged after 3 days, 1, 2 or 4 weeks). The intragroup comparison p values are plotted above the bars.

Discussion

ASNC is a simple and well-tolerated procedure and it makes it possible to obtain important information concerning the diagnostic, prognostic and pharmacological aspects of allergic inflammation. Therefore, this model has been extensively used in clinical and experimental studies. Although the priming effect is a well-known and well-described phenomenon, no information is available concerning the persistence of this effect and in general about the kinetics of the events subsequent to allergen challenge. An analysis of the literature provides sometimes controversial data, and it is difficult to compare different studies, since different interval periods and methodologies of rechallenge have been employed.

The present study evidences some phenomena. (1) The well-known 'priming effect' is detectable by a rechallenge after a 3-day period. (2) The 1-week interval period shows an 'indifferent' phase: the reactivity is equal to baseline. (3) After 2 weeks, such a tolerance is demonstrable: in fact, the target organ evidences a 'hypo-responsive' phase. (4) After 4 weeks, an 'indifferent' phase is present again.

The hyperreactive phase observed here may be due to the presence of inflammatory cells surviving locally since the

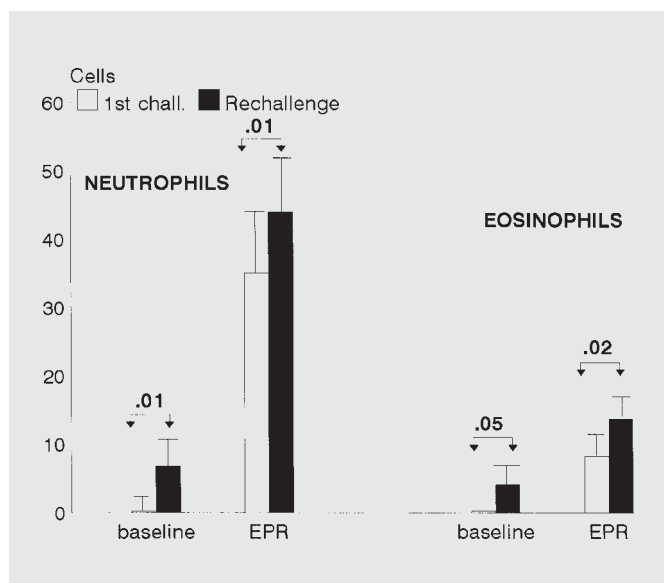


Fig. 4. Eosinophils and neutrophils counts at baseline and 30 min EPR after the first and the second nasal-specific challenge in the group of patients rechallenged 3 days after the first challenge. A significant presence of inflammatory cells is present at baseline before the second challenge. The intragroup p values are plotted above the bars.

first challenge: in fact they are detectable before challenge. In other words, it is conceivable that the persistent inflammation, which is still present 3 days after previous allergen challenge, accounts for the more intense response to rechallenge. This phenomenon is superimposable to that previously demonstrated challenging patients with mite allergy [3]: they showed a threshold-provoking dose lower than subjects with pollen allergy, i.e. the former are more reactive than the latter [3]; this confirms the presence of a 'priming'. This fact is explained by considering that mite allergens are perennial, thus mite-allergic patients are continuously exposed and a persistent inflammation occurs [3]. This persistent inflammation induces a hyperreactivity, higher than in pollen-sensitive patients outside the pollen season.

In addition, only mite-allergic patients with a clinical late phase show an eosinophil infiltrate at baseline and a more intense clinical and inflammatory response in comparison with patients without a clinical late phase [3]. Moreover, mite-allergic patients show a nonspecific hyperreactivity in comparison with pollen-sensitive patients studied outside the pollen season. All these events taken together underline the crucial role played by allergic inflammation in inducing hyperreactivity, both specific and nonspecific.

Recent studies have shown that local nasal immunotherapy is also able to modify the nasal inflammatory events such as the subcutaneous immunotherapy [12]. A possible relationship between subsequent 'hyporesponsive' phases and the effectiveness of local nasal immunotherapy should be investigated. The data of the present study provide two major

pieces of information: the first details the kinetics of the inflammatory response to an induced allergen exposure such as nasal challenge, and the second suggests that when designing a study of rechallenge a proper interval has to be chosen.

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