

REVIEW

Repeated allergen challenge as a new research model for studying allergic reactions

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Experimental allergen challenge

Allergen challenge testing of the nose and lower airways has been used extensively to study the mechanism of allergen-induced mucosal inflammation. In most techniques aerosolized allergen is inhaled through the nose or mouth for short periods of time, in increasing concentrations, until the desired response is obtained.

Experimental allergen challenge of the airways in allergen-sensitive patients often results in an early response, starting within minutes after allergen challenge and reaching its maximum 15–20 min after challenge. In the lower airways in 50–80% of the individuals tested, the early response is followed by a late allergic reaction, usually starting 3–4 h after challenge and reaching a maximum response 7–12 h after challenge. In the nose the reported numbers of late phase responses show large variation between 3 and 75% [1–6], depending on differences in challenge procedures, investigation techniques and positivity cut-offs [7].

Experimental allergen challenge has been used to study the pathology of allergic rhinitis and allergic asthma. Whereas the pathology of the allergen-induced early reaction can mainly be ascribed to the effects of mediators such as histamine, prostaglandins and leukotrienes, the late phase reaction in nose and lower airways is characterized by a cellular infiltrate consisting of eosinophils, lymphocytes, basophils and neutrophils [8–15]. This cellular infiltrate is also characteristic of symptomatic rhinitis [16–19] and asthma [14, 20, 21].

Because of the easy accessibility of the skin, the late phase skin reaction is often used as a model to study inflammatory changes during late phase reactions in the airways. As in the airways, late phase skin reactions are

histopathologically characterized by infiltration of mononuclear cells, basophils, neutrophils and notably eosinophils [22–25].

Experimental allergen challenge in relation to symptomatic disease

Although histopathological changes after allergen challenge resemble the histopathological changes which are observed in symptomatic disease, the presence of a positive challenge reaction does not always coincide with clinical manifestations. Immediate and late asthmatic reactions can be evoked after segmental allergen challenge in rhinitic subjects without a clinical history of asthma [26]. Other studies reported small and insignificant differences between groups with asthma and those with rhinitis in response to allergen bronchoprovocation [27,28]. Muller *et al.* [29] concluded that bronchial responsiveness to allergen bronchoprovocation in allergic individuals had little relationship to the presence or absence of clinical asthma caused by natural environmental challenge.

One of the major limitations of experimental allergen challenge as a research model is the lack of chronicity of the allergen exposure. The chronicity of natural allergen exposure suggests that a continuing allergic reaction may influence further allergen-driven inflammation. In this respect, natural allergen exposure seems to be a better research model. On the other hand, the disadvantage of studying allergic processes in relation to natural allergen exposure is that the allergen load is difficult to quantify.

Repeated experimental allergen challenge

Repeated experimental allergen challenge might serve as an alternative research model to study allergen-induced processes: in this model the chronicity of allergen exposure is imitated, while the allergen load is standardized.

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Studies in the nose

The model of repeated allergen challenge was first used in the nose by Connell in ragweed-allergic patients [30, 31]. When performing daily challenges with ragweed, lower doses of pollen were required on successive days to increase nasal airway resistance. Thus, it was postulated that the reaction threshold was lowered as a result of repeated allergen exposure. The same phenomenon was seen after environmental allergen exposure during the ragweed season [30]. Connell introduced the concept of priming, which is defined as an increased nasal reactivity following repeated allergen exposure. Although Connell's experiments were based on only a limited number of patients, the priming concept is now well accepted. Several years later a number of studies on repeated experimental allergen challenge in the nose were performed and it seems that repeated allergen challenge and priming are not necessarily linked (Table 1).

In 10 ragweed allergic rhinitis patients Wachs *et al.* [32] found an enhanced symptomatic response after three successive daily challenges with the same dose of allergen in six patients, while the other four subjects had little change in responsiveness. Mediator levels (histamine, TAME esterase, kinins and prostaglandin D₂) were significantly increased after the third challenge compared with challenge one and an increased number of eosinophils and neutrophils were found in the nasal lavages 24 h after challenge one and two compared with before. The authors suggested that although there is evidence of priming in the total group of patients, not every subject has a priming response. Those subjects who show priming may become symptomatic during seasonal exposure.

An increased number of eosinophils in nasal lavages 24 h after seven daily challenges compared with the initial challenge was found by Pipkorn, Karlsson and Enerback [33] in birch pollen-allergic rhinitic patients. The increased number of eosinophils remained throughout the whole challenge period, but did not show a further increase after the successive challenges. However, nasal peak flow measurements before and after allergen challenge showed only minor differences between the challenge days. A significant increase in composite symptom score was reached after challenges five, six and seven compared with initial values and tended to decrease after days six and seven compared with day five.

Rechallenge after 10 h in ragweed-allergic rhinitic patients, using a lower dose of allergen, resulted in an enhanced reaction based on mediators in nasal lavage fluid in 21 of 55 subjects [5]. Another 10 subjects showed an enhanced symptomatic response without changes in the levels of mediators.

Repeated allergen challenge with an interval of 48 h, using paper disks, did not result in changes in nasal obstruction, weight of secretion and histamine amount in the secretion in patients with seasonal rhinitis [34].

Whether or not nasal priming occurs seems to depend on several factors. One is the way in which the response is registered. The strongest evidence for priming comes from observations on changes in mediator levels and inflammatory cell numbers in nasal lavages and nasal brushes after repeated allergen challenge. However, it is possible that these changes do not always coincide with physiological or clinical changes.

Table 1. Overview of studies on repeated allergen challenge

Number	Allergen	Interval	No. of challenges	Target organ	Symptomatic response	Response		
						Physiological	Inflammatory	Biochemical
30, 31	Pollen	24 h	4	Nose	n.d.	ER ↑	n.d.	n.d.
32	Pollen	24 h	3	Nose	/10 ↑	n.d.	↑	↑
33	Pollen	24 h	7	Nose	↑	ER =	↑	n.d.
5	Pollen	10 h	2	Nose	31/55 ↑	n.d.	n.d.	21/55 ↑
34	Pollen	48 h	3	Nose	n.d.	ER =	n.d.	No change
35	Pollen	24 h	4	Lower airways	n.d.	ER =	n.d.	n.d.
36	Pollen, cat, dog	14 days	2	Lower airways	n.d.	ER =	n.d.	n.d.
37	HDM	14 days	2	Lower airways	n.d.	ER =; LR =	n.d.	n.d.
39	Pollen, HDM	48 h	2	Lower airways	n.d.	ER =; LR =	n.d.	n.d.
41	HDM	48 h	4	Skin	n.d.	ER =; LR ↑	↑	n.d.
43	Pollen	48 h	3	Nose	n.d.	ER =; LR =	n.d.	n.d.
				Skin	n.d.	ER =; LR =	n.d.	n.d.

HDM = house dust mite; n.d. = not done; ER = early reaction; LR = late reaction.

Studies in the lower airway

Only a limited number of studies have used the model of repeated experimental allergen challenge in the lower airways. Four consecutive bronchial allergen challenges in ragweed-allergic patients with asthma did not result in significant changes in early allergic reaction [35]. No significant change in the early response, but a significant increase in late allergic reaction was found by Rasmussen [36], who performed two bronchial challenges in allergic asthma patients separated by an interval of 14 days. These results are in contrast with a study of Bel *et al.*, in which reproducible early and late phase reactions were found after allergen challenges with an interval of 2 weeks [37]. Ihre and Zetterstrom [38] found a significant increase in non-specific airway responsiveness after repeated low doses of allergen, however, there are no data on the effects on allergen sensitivity (Table 1).

The authors recently performed two repeated bronchial allergen challenges with grass pollen or house dust mite in eight asthmatic patients, using a interval of 48 h [39]. No significant changes in early and late allergic reactions were found (Fig. 1). Bronchial histamine challenges were also performed: the first allergen challenge resulted in a significant decrease in PC₂₀ histamine, however, there was no further decrease in PC₂₀ histamine after the second allergen challenge.

From this summary of the literature it can be concluded that repeated allergen challenge and priming are not necessarily linked. The large variety in individual response patterns to repeated allergen challenge possibly needs a more individual approach. As was suggested by Wachs *et al.*, not all patients show a priming response, and those subjects who do may become symptomatic during seasonal exposure [32].

Recent studies on repeated allergen challenge

The authors recently performed a series of studies on repeated allergen challenges in the skin, nose and lower airways [40]. The effect of repeated allergen challenge in skin on early and especially on late allergic responses was first studied as it was speculated that repeated intradermal skin challenges might be a suitable model to study effects of repeated allergen exposure in the airways. Four repeated intradermal allergen challenges with house dust mite in house dust mite-allergic patients were performed: early, late and delayed phase reactions were measured [41]. Significant increases in late and delayed phase reactions were found after 6, 24 and 48 h, especially after challenge three.

To further explore the usefulness of the model, in the next study it was investigated whether the response to repeated allergen challenge could be influenced by allergen immunotherapy [42]. In this study, 10 house dust mite-allergic patients with asthma who were treated with allergen immunotherapy (SQ503) were investigated. Immunotherapy was effective, which was evidenced by a significant reduction in early and late asthmatic reaction and a significant decrease in bronchial responsiveness to histamine after 1 year of treatment compared with before. After immunotherapy, all patients showed a diminution of the late skin response after repeated intradermal challenge, whereas before immunotherapy 7/10 patients showed priming responses of the late skin reaction after repeated challenges (Fig. 2). So it seems that down-regulation of the late skin response can be achieved by successful immunotherapy and this might give additional information about the handling with chronic or repeated allergen exposure after treatment.

To study whether repeated intradermal allergen challenges might be a suitable model to evaluate effects of

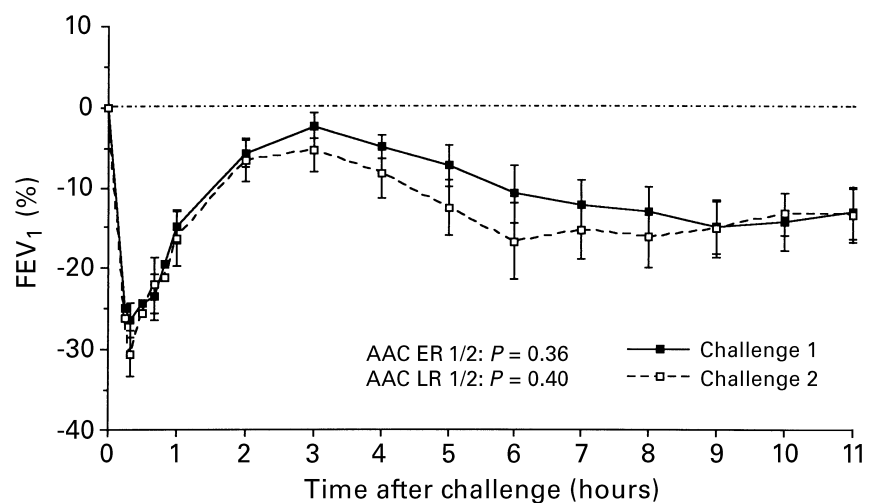


Fig. 1. The effect of repeated allergen challenge in the lower airways using the same dose of allergen and with an interval of 48 h ($n = 8$). AAC ER: area above the curve of the early reaction. AAC LR: area above the curve of the late reaction.

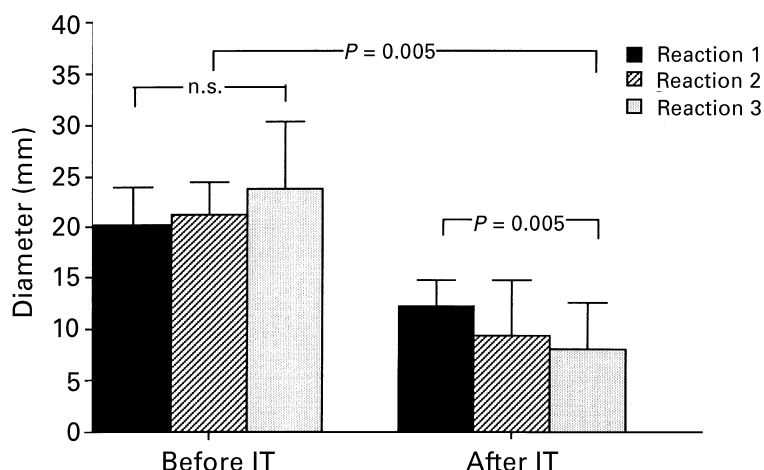


Fig. 2. The effect of allergen immunotherapy on the late skin reaction after repeated allergen challenge ($n = 10$). IT = immunotherapy.

allergen exposure in the airways, repeated allergen challenges in the nose and skin were performed in 11 grass pollen-allergic patients [43]. Three allergen challenges were performed with the same dose of allergen with an interval of 48 h; the effects of challenges one and three were compared. Because the authors were especially interested in late phase responses, a new, very sensitive technique was developed to record nasal reactions after allergen challenge. For this purpose a whole body plethysmography was used [6]. Large differences in response patterns to the repeated challenges were found in the nose and the skin, especially concerning the late response, varying from strongly enhanced responses to complete diminution of the response. For the late response a significant correlation was found between the response pattern to repeated allergen challenge in the nose and the skin (Fig. 3), suggesting that the response to repeated allergen challenge is not just a random variation.

What determines the response pattern to repeated allergen challenge?

As was mentioned above, repeated allergen challenge can result in a wide variation in response. The response pattern (especially late response) may, for example, depend on patients' characteristics, the level of allergen exposition and the type of allergen.

Patients' characteristics: relationship with symptomatic disease

As was stated earlier, the presence of specific IgE or a positive skin test does not distinguish between symptomatic and asymptomatic disease. Patients with a priming response after repeated allergen exposure may have a symptomatic disease, while diminution or down-regulation of the (late) allergic responses in nose and skin after repeated allergen

exposure, which the authors found in a number of the grass pollen-allergic patients, suggests that in some patients the allergic response is self-limiting, possibly resulting in asymptomatic disease.

A significant reduction in symptoms scores despite an unaltered level of specific IgE can be achieved by allergen immunotherapy [44, 45]. The finding that nearly all patients who were successfully treated with allergen immunotherapy showed down-regulation of the late skin response after repeated allergen challenge, suggests that a change in handling with chronic allergen exposure may be responsible for the reduction in symptoms, despite an unaltered specific IgE.

In summary, it appears that some patients in these studies, especially grass pollen-allergic patients, have a natural potential to down-regulate their late response after repeated allergen exposure, while in other patients down-regulation can be induced by systemic administration of high doses of allergen.

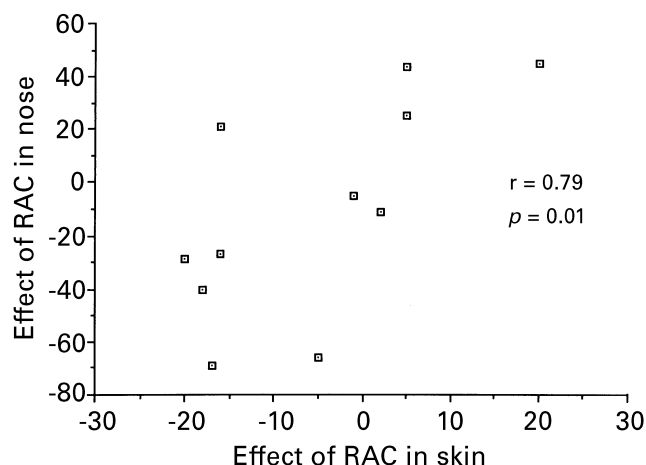


Fig. 3. Correlation between the effect of repeated allergen challenge (RAC; challenge 3 – challenge 1) in the nose and the skin for the late allergic reaction ($n = 11$).

Amount of allergen delivered, dose scheme

The occurrence of priming, or down-regulation, might be dependent on the allergen dose or dose schedule that is used in the repeated challenges. In the authors' study on repeated allergen challenge in nose and skin, a correlation between the allergen dose and the response pattern to repeated allergen challenge could not be found. However, a significant inverse correlation between the reaction strength of the first challenge and the response to repeated allergen challenge was found, suggesting that a rather strong reaction is necessary to induce down-regulating mechanisms.

Apart from the allergen dose used for the challenge, reaction patterns to repeated challenges might be determined by the dose scheme that is used. A decrease in allergen dose on successive challenges, as in Connell's experiments, may lead to priming. On the other hand, repeated allergen challenges with increasing allergen doses, as is used in local nasal immunotherapy, can result in a decreased nasal allergen sensitivity [46–49] and a significant reduction in inflammatory cells in the nose [48].

The type of allergen

Whether or not priming occurs may also depend on the type of allergen involved. In studies performed by the authors, repeated intradermal allergen challenge with house dust mite more often results in an increase in late skin reaction than repeated grass pollen challenges. A possible explanation for this difference in response pattern is that grass pollen-allergic patients were tested outside the season, while house dust mite allergic patients were exposed to allergen during the tests.

Another difference between the two groups of patients was that the house dust mite-allergic patients were all symptomatic, while the grass pollen patients were recruited based on positive skin tests and not on symptomatic disease. A number of patients in this study experienced little or no symptoms during the grass pollen season.

Mechanism underlying priming or down-regulation

The mechanisms underlying priming and down-regulation are still unclear. Experimental nasal allergen challenge results in an increase in inflammatory cells in nasal washings [8,50] and biopsies [10,51] during late phase responses and this cellular influx might serve as a connection between late responses and rechallenge responses [5]. Down-regulation of the response could be explained by an exhaustion of mediators or activation and attraction of inflammatory cells with suppressive characteristics. An appropriate model to study down-regulation after repeated allergen challenge seems to be the repeated intradermal

challenges after immunotherapy. In the authors' study the change in late response pattern after repeated intradermal challenge after immunotherapy coincided with an increased percentage of CD8⁺ cells and CD25⁺ cells in skin biopsies taken 24 h after the first challenge. These CD8⁺ cells might have a potential role in the suppression of the subsequent allergic reaction by production of cytokines such as IFN γ and IL-2.

Summary

Repeated allergen challenge might be a more relevant model for studying symptomatic disease, because it gives more information on the patient's handling of chronic allergen exposure. Thus, this experimental model has more resemblance to the situation of natural allergen exposure, and the allergen load can be standardized. However, the response to repeated allergen challenge in individual patients can show a large variation, that is from a strongly enhanced response to complete diminution of the response. Successful allergen immunotherapy can change the response pattern of repeated allergen challenge in the skin into down-regulation of the late reaction. Chronic or repeated allergen exposure may result in an enhanced allergen-specific allergic response, involving allergen-specific T-cell activation. Different subsets of T cells can exert either activating or suppressive effects on inflammatory cells involved in subsequent allergic reactions. CD8⁺T cells might exert suppressive effects, because they seem to be associated with a subsequent down-regulation of the late skin reaction after repeated allergen challenge (Fig. 4).

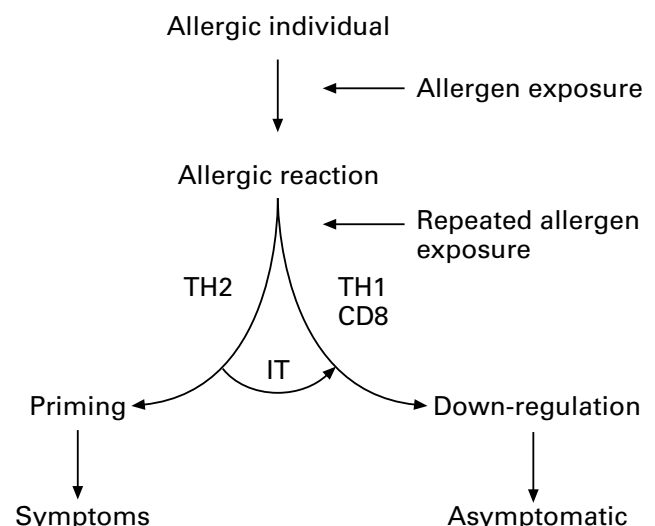


Fig. 4. Different effects of repeated allergen challenge on the allergic reaction.

Further studies are needed to compare the responses to repeated allergen challenge with the response to natural seasonal allergen exposure in the same patients and to explore possible underlying mechanisms using, for example, nasal biopsies.

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