

Review article

Nonspecific provocation of target organs in allergic diseases: EAACI-GA²LEN consensus report

It is widely accepted that nonspecific tissue reactivity is a distinct pathophysiological hallmark of allergic diseases, influenced by genetic and environmental factors different from those involved in causing sensitization and allergen response of target organs. This consensus document aims at reviewing procedures currently used for nonspecific provocation of the bronchi, nose and eye and for measuring their responsiveness to nonspecific stimuli.

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Increasing consensus is being achieved about the systemic nature of allergy (1, 2). But why some patients have only asthma or rhinitis or conjunctivitis and others an association of these diseases in spite of a common generalized hypersensitivity?

Certainly, the route, the type and the quantity of allergen exposure may influence the preferential manifestations of allergy (3). In fact, in patients with a single disease, other organs may react to specific provocation when challenged with adequate dose of allergen (4).

However, it is also conceivable that the reactivity of target organs may influence the clinical manifestations of a systemic sensitization, independently from the homing of allergic inflammatory cells. In fact, it is widely accepted that nonspecific tissue reactivity may be considered as a distinct pathophysiological hallmark of allergic diseases, which is influenced by genetic and environmental factors different from those involved in causing sensitization and allergen response of target organs (5, 6).

Although specific and nonspecific hyperreactivity may influence each other, it is very useful for both pathophysiological and clinical studies as well as for the clinical management of allergic diseases to have tests exploring the nonspecific (nonallergen-induced) responsiveness of target organs. This responsiveness – as implied by the term itself – is not an independent variable, but may differ in relation to the agents used for eliciting response and their specific mechanism of action. In fact, it is well known that responsiveness to different stimuli does not necessarily overlaps.

While nonspecific bronchial provocation is widely used, and tests are standardized enough, the pathophysiological and clinical usefulness of nasal and conjunctival tests still waits for more accurate evaluation and diffusion.

This consensus document aims at reviewing procedures currently used for the nonspecific provocation of the bronchi, nose and eye and for measuring their responsiveness to nonspecific stimuli.

Nonspecific bronchial provocation

Rationale

Airway hyperresponsiveness is regarded as a characteristic feature of bronchial asthma, but it may also be present in other allergic or nonallergic respiratory disorders (7, 8). Different stimuli are currently used to test for airway hyperresponsiveness. Based on their mechanism of action, they are categorized as direct or indirect stimuli. The former cause airway smooth muscle contraction by activating specific receptors on its cell membrane, the latter through the release of mediators from intermediary cells (9). Common examples of indirect stimuli are exercise, dry air, hypertonic or hypotonic solutions, adenosine, mannitol and sensitizing agents (Table 1.).

Table 1. Tests for assessment of nonspecific bronchial reactivity

Direct
Methacholine
Histamine
Indirect
Exercise
Dry air and eucapnic voluntary hyperpnoea
Hypertonic or hypotonic solution
Mannitol
Adenosine and cAMP

Among the direct stimuli, methacholine (MCh) has been the most widely used for both clinical and epidemiological purposes (10) and is currently the only pharmacological agent approved by authorities in most countries for use by inhalation in humans. MCh mimics the effect of endogenous acetylcholine on airway smooth muscle muscarinic receptors of the M_3 subtype (11). It has the advantage of being much more resistant to degradation by cholinesterase, which allows obtaining cumulative dose–response curves (12). An alternative bronchoconstrictor agent is histamine, but its use is limited by the occasional occurrence of side effects (headache, sore throat, itching) and because of less reproducibility (13, 14). Apart from these differences, which give certain advantage to MCh for clinical use, the two direct bronchoconstrictors are almost equivalent on a molecular basis.

Among the indirect stimuli, exercise challenge was first used for assessing airway responsiveness, mainly in children, in whom exercise may be a prominent cause of the symptoms (15, 16). Guidelines for the standardization of exercise challenge have been recently published (17, 18). A major advantage of this test is its specificity, as normal subjects do not respond. However, its sensitivity is less than that of MCh, and many asthmatic patients do not react to this stimulus, which is a major limitation for its clinical or epidemiological use (19–21). Moreover, exercise challenge must be performed in conditioned environment, as response is highly dependent on air humidity and temperature (22). Dry-air isocapnic hyperventilation can be used as a surrogate of exercise challenge (23), but apart from the required technical sophistication, this approach would not improve the sensitivity of the method and no guidelines for its standardization have been worked out so far. Other surrogates of exercise challenge use hypertonic solutions (24, 25), or mannitol, which can be easily inhaled from a powder-delivering device (26). Adenosine and its more soluble derivative cyclic adenosine monophosphate (cAMP) have also been used as indirect stimuli for inhalation challenge in asthma (27, 28). They cause mast cell degranulation (28, 29) by activating specific mast cell receptor A2B (30). A dose–response curve as for MCh can be obtained.

As already hinted above, the major difference between direct and indirect stimuli for the diagnosis of asthma is believed to be a greater sensitivity of the former and a greater specificity of the latter. Indeed, some indirect

stimuli have been shown to correlate better with indices of airway inflammation than MCh (31). In this context, for example, bronchial challenge with AMP was reported to distinguish children with asthma from those with cystic fibrosis, bronchiectasis and bronchiolitis, whereas hyperresponsiveness to MCh was present in all these conditions (32). The disadvantage of using more specific stimuli is the loss of sensitivity and the risk of inducing airway inflammation, as clearly demonstrated for allergen (33) and adenosine (34) challenges. In contrast, direct stimuli are sensitive to changes in the mechanical properties of airways and lung parenchyma, independently of the presence of inflammatory cells in the airways at the time of the challenge. It is, therefore, possible that asthmatic subjects with little inflammation but significant airway remodelling are hyperresponsive to direct stimuli but do not respond to indirect stimuli. However, it is also possible that allergic subjects who never had asthma do respond to indirect stimuli because of the presence of inflammatory cells in their airways, without being hyperresponsive to a direct stimulus (35).

Direct bronchial challenge tests

Methacholine challenge. There is wide agreement that MCh challenge is helpful for the diagnosis of asthma in subjects with a history of symptoms as wheezing, dyspnoea, chest tightness, cough upon exposure to allergen, cold air breathing, in work environment or during respiratory infections, in whom baseline spirometry is normal (36). Indeed, the optimal diagnostic value of the MCh test is when the above-mentioned symptoms are present and when a pre-test probability of asthma ranges between 30% and 70% (37). This requirement is dictated by the necessity to compensate for the low specificity of the test. Indeed, due to the complexity of the mechanisms of airway narrowing, the MCh challenge may be positive in diseases other than bronchial asthma, such as chronic obstructive pulmonary disease (COPD) (8), congestive heart failure (38), sarcoidosis (39), cystic fibrosis (40), bronchiectasis (41) and allergic rhinitis (7, 42). In this last disease, however, a positive challenge may be suggestive for the development of asthma, mainly when the maximal response is assessed (42). Furthermore, once the diagnosis of asthma has been established, the MCh challenge may be valuable for optimizing the pharmacological treatment (43–45) and for monitoring changes of airway responsiveness that may worsen during exacerbations due to exposure to allergen (33) or upper airway viral infections (46), or may improve after proper treatments (44). On the other hand, the strength of the MCh challenge is its high negative predictive values, as a negative response is very unlikely to occur in current asthmatic subjects, thus making it valuable in excluding the diagnosis of asthma (47).

The use of MCh for bronchial challenge has been proven to be safe, and can be done in a majority of

patients (17, 18). It is absolutely contraindicated only in patients with severe airflow obstruction ($FEV_1 < 50\%$ of predicted or < 1.0 l), 3 months after heart attack or stroke, uncontrolled hypertension (SBP > 200 , or DBP > 100 mmHg), or aortic aneurysm. Relative contraindications include moderate airflow obstruction ($FEV_1 < 60\%$ of predicted or < 1.5 l), inability to perform acceptable quality spirometry, pregnancy, breast feeding, current use of cholinesterase inhibitors (e.g. for myasthenia gravis), cholinergic urticaria.

Indirect bronchial challenge tests

Exercise challenge. Some asthmatic subjects develop exercise-induced bronchoconstriction (EIB), and this may be the unique manifestation of childhood asthma (15, 16, 48). During exercise, increased minute ventilation dries up the airway mucosa, causing hyperosmolarity of epithelial lining fluid (49). This is a strong stimulus for mast cell degranulation (50) and bronchoconstrictive reflexes. Normally, the increased tidal volume during the exercise (51), together with the increased sympathetic tone, would cause bronchodilatation and prevent the development of EIB after it. When bronchoconstrictor mechanisms prevail over protective mechanisms, EIB develops.

Hyperosmolarity causes release of mediators from mast cells (50), which, in turn, activate specific receptors of airway smooth muscle, leading to bronchoconstriction. Results of pre-treatment studies (52) suggest an important role for leukotrienes. Prostaglandin E_2 is considered the main determinant of refractoriness to a second exercise bout, which may last as long as 4 h (53). Indeed, in a majority of subjects with EIB a second exercise causes no or less bronchoconstriction than that induced by the first exercise. Pre-treatment with COX₂ inhibitors as indomethacin blunts refractoriness (54).

Exercise test for investigating EIB is indicated for childhood asthma, as well as for sport and occupational medicine. A conditioned environment is needed, as temperature and relative humidity of the air are crucial determinants of osmotic changes. Temperatures of 20–25°C and relative humidity of $< 50\%$ (< 10 mg water/l) are recommended. An exercise may be performed on a treadmill or a veloergometer, the latter allowing a better support of the ventilation valve or mask and making the workload independent of body weight (18, 55, 56).

The response is considered positive for the occurrence of EIB, when FEV_1 falls $\geq 10\%$ between 5 and 30 min after the end of the exercise. Late falls of FEV_1 have been observed, but their link to the exercise challenge is uncertain (57). Contraindications for exercise challenge are the same as for MCh challenge, but greater attention is to be paid to cardiovascular diseases and cardiac arrhythmia.

Dry air challenge. As EIB is mainly due to hyperpnoea-induced osmolar changes of airways, bronchoconstriction

can be alternatively induced in susceptible subjects if they voluntarily increase minute ventilation while breathing dry air from a reservoir bag without exercising (58–60). To avoid hypocapnia and alkalosis, the air must be supplemented with CO₂ (5%). There is no agreement whether dry cold air is more effective than air at the room temperature, as the effect of cold on airway calibre has not been fully established. Monitoring of minute ventilation is strictly needed. Furthermore, airway response may be variably affected by the pattern of breathing used to increase ventilation, i.e. whether increasing tidal volume or frequency, but no information is currently available. Both incremental and single-bout protocols have been proposed, as no guidelines have been worked out so far. Eucapnic voluntary hyperpnoea (EHV) has recently been reported as a good alternative to exercise challenge, particularly in sports medicine (23, 58, 59).

Hypertonic solution challenge. As hyperpnoea-induced bronchoconstriction is believed to be due to an increase in the osmolarity of the airway lining fluid, an alternative approach to provoke it would be to use the inhalation of hypertonic solution (61–63). An ultrasonic aerosol generated from a 4.5% saline solution is inhaled during spontaneous tidal breathing. The exposure time is progressively increased from 30 s by double increments until the FEV₁ falls by 10% or more. This test is indicated in children because minimal cooperation is required.

Mannitol challenge. Inhalation of mannitol has been recently proposed by Anderson and co-workers (26) as a surrogate of eucapnic hyperventilation or hypertonic solution tests. Mannitol is an indirect, osmotic stimulus that causes, like exercise, airway mucosal hyperosmolarity, water loss and bronchoconstriction in susceptible subjects. Powder mannitol is inhaled from gelatine capsules containing 5, 10, 20 or 40 mg to a cumulative dose of 635 mg. Lung function is monitored during the challenge and PD₂₀ is calculated. Agreement between mannitol challenge, eucapnic hyperventilation and exercise seems good (64) and the sensitivity and specificity of this kind of challenge for asthma, in comparison with the other traditional challenges, are under investigation.

Hypotonic solution challenges. Ultrasonically nebulized distilled water (UNDW) has also been proved to elicit bronchoconstrictor response in asthmatic patients and cough alone in some healthy subjects (65). The technique has been further modified and standardized, and speculations on its mechanisms have been proposed (66). The dose triggering airway narrowing in most asthmatic patients is <2 ml of UNDW (67). A positive response to UNDW is more likely when PD₂₀ MCh is <2 µmol, but the overall correlation with these direct challenges is poor. Bronchial response to UNDW correlates poorly with MCh responsiveness (68). The degree of bronchial

responsiveness to UNDW is in good concordance with the response to exercise and to eucapnic hyperpnoea (61). Ultrasonically nebulized distilled water is broadening the notion of the importance of nonisotonic stimuli for altered airway responsiveness.

Factors affecting airway responsiveness

The variability of responses to different types of bronchial challenges can be reduced by controlling the factors that may decrease or increase it (ref. ATS). The former include either drugs (β-agonists, theophylline, anticholinergics, steroids, cromones, antileukotrienes) and foods (coffee, tea, cola drinks, chocolate). The latter include exposure to various environmental agents (allergens, occupational sensitizers, air pollutants, chemical irritants, smoke), recent respiratory infections and β-blocking drugs. Drugs affecting the contractility of airway smooth muscle should be discontinued before the tests for a specific time period, depending on their half-lives. Regularly taken controller drugs (e.g. steroids) should not be withdrawn for testing purposes, but the kind and dose of the preparation used should be taken into account in the interpretation of the results. Testing after exposure to environmental agents or a respiratory infection is appropriate if their effect on airway hyperresponsiveness is to be evaluated.

In addition to the above factors influencing airway smooth muscle contractility or airway inflammation, the response to bronchoconstrictor stimuli, mainly to the direct ones like MCh, may be greatly affected by the effect breathing manoeuvres, either in relation to the inhalation the bronchoconstrictor agent or to the measurement of lung function (69). Deep breaths in healthy subjects attenuate the induced bronchoconstriction, whereas in asthmatic subjects this beneficial effect is blunted or absent (70). Even though the mechanism of the effect of deep breaths on airway calibre are still under intense investigation, a practical inference can be made that FEV₁, which requires full lung inflation, discriminates nonasthmatic from asthmatic subjects better than other spirometry indices not requiring full lung inflation, such as airway conductance (69). The latter, however, can distinguish between rhinitic and asthmatic subjects better than FEV₁ (69). Therefore, the accuracy of MCh challenge, as of any other bronchial challenge, is greatly dependent on the strict adherence to standardized procedures, in which the mode of inhalation as well as the number and timing of FEV₁ measurements are kept constant (71).

Concluding remarks

Different challenge methods have been proposed for assessing airway hyperresponsiveness. Those using direct stimuli on airway smooth muscle are regarded as more sensitive, while those acting through mediator release are considered more specific for the diagnosis of bronchial

asthma (72) and for assessing the efficacy of anti-inflammatory treatment (72, 73). Airway hyperresponsiveness is not unique to bronchial asthma, but a combination of two or more methods for its assessment may be useful to discriminate it from other respiratory disorders.

It is now recognized that airway smooth muscle abnormal response and remodelling are the underlying mechanisms for increased response to bronchoconstrictor stimuli, and mediator release represents only a trigger factor in this process. Subsequently, the most appropriate way of assessing abnormal responsiveness of the target organ is by using a direct stimulus acting on the airway smooth muscle, such as MCh. The use of indirect challenges may be useful in specific circumstances, but interpretations of the level of airway responsiveness based on them should also take into consideration the inflammatory phenomena going on at the time of the test.

Nonspecific nasal provocation

Rationale

Nonspecific nasal hyperreactivity is an abnormal reaction of the nasal tissue to stimuli which are usually innocuous for most persons (74). The excessive naso-sinusal response to heterogeneous stimuli is caused by a dysregulation of the autonomic nervous system at various levels of the neurovegetative reflex arc in the nose (75):

- 1 increase of permeability of the nasal mucous membrane to irritant stimuli;
- 2 increase of mastocytic, eosinophilic and neutrophilic infiltration;
- 3 decrease of the stimulation threshold of eosinophils, neutrophils and mastocytes;
- 4 excessive excitation of trigeminal afferent fibres and
- 5 alterations of the central control of the reflex arc.

Knowledge of the pathophysiology of the neurovegetative system has shown the complexity of relationships among the numerous neurotransmitters involved (Table 2) and the specific and nonspecific stimuli which are responsible for nasal hyperreactivity.

Despite differences in the pathophysiology of specific and nonspecific hyperresponsiveness, symptoms induced

Table 2. Neurotransmitters involved in the neurovegetative reflex arc in the nose

Acetylcholine at parasympathetic postganglionic synapses level (in secretion granules)
VIP (Vasoactive Intestinal Peptide) at parasympathetic postganglionic synapses level of vessels, mucoserous glands, smooth muscle fibres, subepithelial layer
PHI (Peptide Histidine Isoleucine) parasympathetic postganglionic synapses
SP (substance P) amyelinic trigeminal endings
NKA (Neurokinine A) amyelinic trigeminal endings
NPY (Neuropeptide Y) amyelinic trigeminal endings of vascular nerve endings and of smooth muscle fibres
CGRP (Calcitonin Gene-related Peptide) amyelinic trigeminal endings

by specific and nonspecific challenges are almost superimposable, being essentially represented by nasal obstruction, rhinorrhea and sneezing. Such symptoms are the result of the protective reflexes of the nasal mucous membrane in order to remove irritant particles or substances (75).

While the allergen challenge of the nose is standardized enough, at present methods for studying the nonspecific nasal reactivity differ from centre to centre (76). As a matter of fact, in routine rhinologic clinical practice, the diagnosis of nonspecific nasal hyperreactivity is often made by the exclusion of sensitization through skin tests, immunoglobulin E determination and Active Anterior Rhinomanometry (AAR)/Acoustic Rhinometry (AR) after Specific Nasal Challenge Test (SNCT; 77).

The aim of this document was to discuss the advantages and limits of nonspecific nasal challenge tests.

Nonspecific nasal challenge test

Several stimuli are capable to induce nonspecific nasal responsiveness, but a selective and reliable test to measure it is still missing.

The characteristics of an ideal test should be to:

- 1 able to reproduce rhinitis symptoms;
- 2 able to detect changes over time;
- 3 allow documentation through objective measures;
- 4 be reproducible and
- 5 be sensitive and specific enough.

Cold air test

Method. The test with cold stimulation is carried out through nasal instillation of cold water drops (0–5°C) or through the breathing of dry and cold air or administration through spray at a flow of 26 l/min, a temperature of 0–5°C and a Relative Humidity rate (RH) < 10% (78). The evaluation of response to stimulation is made through AAR, carried out at basal conditions and following challenge.

Mechanism. Cold induces the vaporization of water and fluids from the mucous membrane resulting in changes of osmolarity which reduces the release of mediators (histamine, PGD₂, kinins). It has been suggested that cold also induces a direct neurogenic stimulation of the cholinergic system (78).

Advantages. 1 Easily performed. 2 Good tolerability by the patient.

Limits. 1 The increase of resistances is transient and not higher than 15% on an average. 2 It is nonsensitive enough, as it does not provide significant changes of nasal resistances also within homogeneous groups. 3 It is not specific enough.

Methacholine test

Method. The test is carried out through the administration of increasing doses of MCh (3–6–12–24–48 mg diluted in 0.4 ml of phosphate buffer solution) followed by the measurement of secretions collected in a paper handkerchief 15 min later (79). At high doses, MCh causes nasal hypersecretion in both rhinopathic and healthy subjects. The provoking dose of 6 mg of MCh in 0.4 ml of solution, usually allows to discriminate between hyperreactive subjects and normoreacting ones.

Mechanism. Methacholine is an acetylcholine-like substance. Cholinergic receptors of the nasal mucous membrane are found in correspondence of glandular acini but not at the vascular level. This explains the clinical finding of hypersecretion associated with minimal nasal congestion.

Advantage. Good index of mucosal reactivity (bronchial and nasal).

Limits. 1 It does not evoke significant changes of nasal resistances. 2 Difficult to discriminate between specific from nonspecific rhinitis.

Histamine test

Method. The method is based on the administration through an aqueous solution spray of 1–2 mg/ml of histamine. Lower doses of 0.5 mg/ml cause considerable effects only in rhinopathic subjects with a prevailing obstructive component ('blockers'; 80). The objective evaluation of changes induced by histamine is based, in addition to the evaluation through the objective rhinologic examination of rhinorrhea entity and the number of sneezes, on AAR which gives objective data on nasal resistances.

Mechanism. Histamine is the most potent mediator in both specific and nonspecific vasomotor responses. It acts both directly at the mucosal level and through nervous reflexes on vessels and glands. Clinically, it causes prevalently oedema and consequently nasal congestion.

Advantage. A good marker of nasal vasomotor response and neuroreflex.

Limit. It does not evoke significant modifications of glandular secretion.

Phentolamine test

Method. The method consists of the nasal instillation through the spray of 0.200 mg of substance per naris. Also in this case, the evaluation through rhinomanometry represents the most objective method to evaluate

response through the measurement of nasal resistances (81).

Mechanism. Phentolamine is a potent α -1-blocker which promotes the paralysis of autonomic regulation of the capillary bed. Such modification occurs exclusively in patients with a defect in the α -adrenergic system, occurring in chronic rhinopathy.

Advantage. It causes a significant increase in nasal resistance in allergic subjects.

Limit. Poor diagnostic value. The drug induces nasal reactivity only in the delayed phase of nasal pathology, when the autonomic nervous system has been compromised. It does not allow to identify hyperreactivity conditions in the initial stage.

Capsaicin test

Method. The application of nasal drops of capsaicin (8-methyl-N-vanillyl-6-nonenamide) extracted from capicum induces responses of the nasal mucous membrane due to its selective action on type C amyelinic fibres. The evaluation of responses is assessed through the symptom score (obstruction, rhinorrhea, sternutation) and AAR.

Mechanism. Capsaicin is thought to cause a direct stimulation of nervous fibres generating an action potential. Furthermore, it is able to cause an increase in vascular permeability and to produce mucous hypersecretion through the release of neuropeptides (SP, NKA).

Advantage. Some authors consider the test suitable to differentiate subjects with nasal hyperreactivity from normal subjects as well as allergic from 'aspecific' rhinopathic 'sneezers' (82).

Limits. 1 Of difficult clinical application. 2 Nonspecific. 3 Scarcely repeatable or standardizable.

Ultrasonic nebulized distilled water test

Method. It consists of the inhalation of distilled water hypotonic solution in a monodiffused phase, delivered through a nasal adapter for 30–60 s. The evaluation of the nasal response is assessed through the determination of nasal volumes by means of AR and of nasal flow by means of AAR.

Mechanism. As at the bronchial level where the test is widely used, it is believed that UNDW causes a dilution at the intraluminal epithelial surface level. This is caused by the escape of water from epithelial cells with their recall of Cl^- ions from the vascular bed and Ca^{++} and Na^+ from the cellular apical side.

Such process brings to the generation of a transepithelial gradient with increase of cytoplasmic Ca^{++} , which eventually binds to calmodulina and Ca-tropoin with the production of kinins and other mediators (81).

Advantages. **1** Absolute absence of side effects. **2** Active participation is not needed (the test can be easily carried out in children <5 years). **3** It is able to cause a significant increase in nasal resistance in rhinopathic subjects (atopic and nonatopic). **4** It detects nasal hyperreactivity at an early stage.

Limits. **1** Difficult to distinguish between specific and nonspecific hyperreactivity. **2** Further studies are needed to understand mechanisms of action.

Concluding remarks

Methods for studying nonspecific nasal reactivity still appear to be not specific, sensitive and standardized enough. Moreover, nonspecific hyperreactivity recognizes various mechanisms not yet perfectly known. The clinical heterogeneity of rhinitics calls therefore for the 'personalization' of tests, taking into consideration the prevailing symptoms (nasal obstruction, rhinorrhea, sneezing) in individual cases. Among the methods discussed above, the histamine test associated with the MCh test may represent a reliable combination to study nonspecific vasomotor response. The promising results obtained with ultrasonic nebulization and the lack of side effects suggest a wider diffusion of this method. A more systematic use of the nonspecific nasal provocation may help for a better understanding of this pathogenic entity and to avoid that it continues to represent an 'exclusion diagnosis'.

Nonspecific conjunctival provocation

Rationale

The conjunctiva is a mucous membrane that covers the inner surface of the eyelids and folds back to cover the front surface of the eyeball extending to the edge of the cornea, the limbus. As it is transparent, blood vessels are easily visible underneath it. The conjunctiva is composed of two layers: the conjunctival epithelium and the stroma. The conjunctival epithelium is from 2 to 10 cell layers' thick. Within the conjunctival epithelium, goblet cells are found. These cells secrete mucins, an important component of the pre-corneal tear film layer that protects and nourishes the cornea. It also represents an active barrier to airborne pathogens and allergens, and plays an important role in the cellular immune response (83). The stroma of the conjunctiva is made of two layers: the outer one containing loose connective tissue rich of lymphoid tissue, mast cells and neutrophils; and the inner

one (fibrous layer), containing elastic tissue, blood vessels, lymphatic vessels, nerves and Krause's glands. The conjunctiva is innervated from sensory, sympathetic and parasympathetic nerves several neurotransmitters (substance P, CGRP, VIP, NPY; 84, 85).

Allergic conjunctivitis may be classified on the basis of the clinical presentation in seasonal allergic conjunctivitis (SAC), perennial allergic conjunctivitis (PAC), vernal keratoconjunctivitis (VKC), atopic keratoconjunctivitis (AKC) and giant papillary conjunctivitis (GPC) (83). As the conjunctiva is a mucosal surface similar to the nasal mucosa, the same allergens that trigger allergic rhinitis may be involved in the pathogenesis of allergic conjunctivitis. Common airborne antigens, including pollen, grass and weeds, may provoke symptoms of acute allergic conjunctivitis, such as ocular itching, redness, burning and tearing.

The existence of a nonspecific hyperreactivity in allergic patients is widely accepted. Several nonallergic stimuli may induce a hyperreactive reaction in the target organs of atopic patients (86, 87). Although close relationships exist between reactivity to allergens and to nonspecific stimuli, specific and nonspecific hyperreactivity represent distinct concurrent mechanisms in the pathogenesis of allergic diseases (88).

In the eye, as in other target organs of allergic reaction, nonspecific conjunctival reactivity is shown to be greatly variable in the same patients around the year and among different subjects affected by the same allergic conjunctivitis. In fact, patients affected by allergic conjunctivitis show a variability of signs and symptoms from day to day and even in the course of a single day. Possibly, the sun, the wind, the dust, cold air or other nonspecific agents only represent triggers of a nonspecific abnormal reactivity of the conjunctiva. However, hyperresponsiveness to nonspecific agents may play *per se* a role in the pathogenesis of allergic conjunctivitis.

The pathogenesis of nonspecific hyperreactivity is not clearly understood. Hyperreactivity of the conjunctiva has been postulated to have a genetic component as for other mucosal surfaces. However, close relationships appear to exist between mucosal hyperreactivity and tissue inflammation (88, 89). In fact, inflammation induced by a variety of stimuli – including bacteria, viruses and allergen challenge inducing late-phase reactions – is associated with bronchial and nasal hyperresponsiveness (90–93). The hypothesis of a relationship between inflammatory cell migration, late allergic reactions and allergen-induced tissue hyperresponsiveness, is supported, in the airways, by the observation that an increase in histamine, MCh and AMP responsiveness, recorded 3 h after allergen inhalation, precedes the late asthmatic response, and that there is a significant correlation between the increase in MCh bronchial hyperresponsiveness at 3 h and the development of late asthmatic reactions (94, 95).

These findings could be regarded as indirect evidence of the contribution of inflammatory cells to allergen-induced increase in nonspecific bronchial responsiveness. As for the lung, it is possible to hypothesize that the inflammation may have a role in conjunctival nonspecific hyperresponsiveness. In fact, the presence of 'minimal persistent inflammation' (i.e. eosinophil and neutrophil infiltrates and intercellular adhesion molecule-1 (ICAM-1) expression on epithelial cells) was demonstrated in symptom-free patients with rhinitis and conjunctivitis due to mite allergy (96, 97). In these patients, conjunctival hyperreactivity to nonspecific stimuli was observed. Therefore, it is possible to hypothesize that the constant exposure to the allergen promotes conjunctival inflammation (96–98).

The links between inflammatory cells and hyperreactivity are probably complex and mediated not only by different inflammatory mediators, such as interleukin-13, but also involve an endocrine and neural control (99). Several studies suggest involvement of neurogenic inflammatory mediators in allergic conjunctival reaction, such as substance P and the nerve growth factor (100–102).

Another potential mechanism of the allergic nonspecific hyperresponsiveness may be related to the damage of the epithelium, thereby facilitating the passage of the provoking substance. In fact, the conjunctival cells in allergic patients seems to be less tightly bound together than healthy epithelia. This means that allergens and nonspecific stimuli could enter the underlying tissues from patients with all forms of allergic reaction. However, some evidence showed that, at least in the lung, there is no relationship between increased epithelial permeability and increased nonspecific tissue responsiveness (103).

Nonspecific conjunctival challenge tests

There are a few studies on nonspecific conjunctival reactivity.

Histamine (0.01–1.0 mg/ml) and hyperosmolar glucose solutions (10–50%) were used for nonspecific conjunctival provocation although other stimuli, such as cold dry air, light or irritating substances, are known to cause a nonspecific neurovascular reaction of the conjunctiva and might be potential candidates as nonspecific agents for eye challenge tests.

In the eye, the redness score of photographs, or other more standardized criteria such as the evaluation of inflammatory cells or cell mediators levels, have been used to quantify the conjunctival responsiveness (96, 104–106).

In guinea pigs, topical histamine challenge has been used to evaluate conjunctival vascular permeability after an immediate ocular hypersensitivity reaction (107).

Histamine conjunctival challenge and hyperosmolar solution have also been used in humans to mimic signs and symptoms of allergic conjunctivitis. In particular,

patients with vernal keratoconjunctivitis or allergic conjunctivitis due to mites demonstrated hyperreactivity to histamine diphosphate, showing conjunctival hyperaemia and chemosis following conjunctival administration (104). Although histamine analyses only one parameter of the ocular response, namely the vascular reactivity of the conjunctiva, it was demonstrated that at the lowest doses tested, this agent discriminated between asymptomatic patients with vernal keratoconjunctivitis and healthy individuals (104). Histamine challenge induces neither an inflammatory response nor CD54 expression on the epithelium of allergic subjects and healthy volunteers (98). On the other hand, a hyperosmolar stimulus (i.e. glucose solution) at the conjunctival level was able to produce an immediate inflammatory response with clinical signs and symptoms, inflammatory cell infiltrates and CD54 expression on the conjunctival epithelium of all allergic subjects and healthy volunteers (96). Thus, histamine and the hyperosmotic solution probably have different mechanisms of action: while histamine is able to induce vascular reactivity only, the glucose solution induces epithelial osmotic variations causing inflammatory cell infiltrates and expression of epithelial adhesion molecules.

It is interesting to note that only house dust mite-sensitive subjects showed conjunctival hyperreactivity to the hyperosmolar solution, while pollen-sensitive subjects out of pollen season and normal subjects required a significantly higher provoking dose to elicit a clinical reaction (96). These findings suggest that conjunctival hyperreactivity may be related to the 'minimal persistent inflammation' present in mite-sensitive subjects but not in pollen-sensitive subjects and related to the continuous exposure to the allergen (97). In fact, at baseline, only house dust mite-sensitive subjects showed evidence of a mild cellular infiltrate and a low, but significant, expression of CD54 on conjunctival epithelium, when compared with healthy subjects and Parietaria J-sensitive patients (96).

While data above mentioned do not allow to define whether conjunctival response to nonspecific stimuli was only a consequence of an allergic inflammation or an independent pathophysiological feature of a 'red eye', a more recent study of subjects with ocular discomfort, with or without allergy, firstly showed that nonspecific conjunctival hyperreactivity may also be documented independently of underlying atopy (108). In fact, a hyperosmolar provocation test with increasing concentrations of a glucose solution (10–50%) was able to discriminate between subjects with or without ocular discomfort in both the allergic and non allergic group. Forty percent glucose was the optimal threshold dose for prediction of conjunctival hyperreactivity with the highest sensitivity and specificity (108). Therefore, it seems that in the eye too nonspecific conjunctival hyperreactivity may represent a distinct pathophysiological hallmark of tissue hyperreactivity even in the absence of allergic inflammation.

Concluding remarks

In the eye, nonspecific hyperreactivity has so far been mainly related to allergic inflammation and to allergen exposure. Therefore, allergen avoidance measures and long-term antiallergic therapy may represent an appropriate strategy for patients with conjunctival hyperreactivity. As allergen exposure cannot be completely abolished, pharmacological approaches have been proposed for the management of the hyperreactive eye (108–112).

Antihistaminic drugs may prevent clinical and cytological changes following specific allergen conjunctival challenge (mainly during the late phase). Cetirizine treatment, acting on the minimal persistent inflammation, reduces ICAM-1 expression on epithelial cells and decreases inflammatory infiltration in patients with allergic rhinitis and conjunctivitis (110, 111). Cetirizine and azelastine seem also able to modulate reduce nonspecific conjunctival hyperresponsiveness to a hyperosmolar conjunctival challenge in patients with asymptomatic mite-allergic conjunctivitis (109–113). Further investigations of conjunctival hyperreactivity occurring even in the absence of an underlying allergic inflammation may increase our understanding of its pathophysiological basis allowing a more accurate diagnosis and hopefully improving therapeutic approaches. Indeed, the anatomical features of the conjunctiva and the accessibility of this tissue render this target organ an ideal model to study the relationships between specific and nonspecific hyperresponsiveness in allergic diseases.

Conclusion

Nonspecific provocation tests are necessary tools for exploring the nonspecific reactivity of organs involved in allergic diseases. For some tests (such as exercise and MCh bronchial challenge), there is enough evidence to allow their use in clinical practice, for others' use should be confined to research purposes. As different stimuli may explore different aspects of tissue hyperreactivity, the choice of the 'best test' will largely depend on the facilities available as well as on the specific aim and application.

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