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REVIEW ARTICLE

Organ-specific allergen challenges in airway allergy: Current utilities and future directions

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Abstract

Atopy has been long used as the screening method for airway allergy. Nevertheless, aeroallergens can trigger respiratory symptoms not only in atopic patients (atopic respiratory allergy, ARA), but also in non-atopic subjects (local respiratory allergy, LRA). Moreover, ARA and LRA can coexist in the same patient, and this clinical scenario has been called dual respiratory allergy (DRA). When the clinical history cannot determine the relevance of sensitizations in ARA patients, nasal, conjunctival or bronchial allergen challenges (NAC, CAC, and BAC, respectively) should be conducted. Moreover, these tests are required to identify patients with LRA and DRA. The clarification of the allergic triggers of airway diseases has a profound impact on the management strategies the patients can be offered. Importantly, allergen immunotherapy (AIT) remains as the only disease-modifying intervention for ARA. Recent data indicate that AIT might have a similar effect on LRA patients. Nevertheless, AIT success relies largely on the correct phenotyping of allergic individuals, and NAC, CAC, and BAC are very helpful tools in this regard. In this review, we will summarize the main indications and methodology of CAC, NAC, and BAC. Importantly, the clinical implementation of these tests might translate into precision medicine approaches and better health outcomes for patients with airway allergy.

KEYWORDS
allergic asthma, allergic rhinitis, bronchial allergen challenge, conjunctival allergen challenge, nasal allergen challenge

Abbreviations: AIT, allergen immunotherapy; ARA, atopic respiratory allergy; BAC, bronchial allergen challenge; BAT, basophil activation test; CAC, conjunctival allergen challenge; DRA, dual respiratory allergy; EAACI, European Academy of Allergy and Clinical Immunology; EAR, early asthmatic response; HDM, house dust mite; ICS, inhaled corticosteroids; LAR, late asthmatic response; LRA, local respiratory allergy; NAC, nasal allergen challenge; OCS, oral corticosteroids; s, allergen-specific (antibodies); SPT, skin prick test.

1 | INTRODUCTION

Airway allergy (allergic rhinitis, conjunctivitis, and asthma) affects over 300 million people worldwide,[1] and accounts for a high burden to society and individuals including both direct (e.g., attendance to emergency room) and indirect (e.g., loss of productivity and impaired school performance) costs.[2,3] Patients with airway allergy suffer from nasal, bronchial, and/or conjunctival allergen-specific reactivity, and the demonstration of this trait is required for a confirmatory diagnosis.[4] In this regard, the nasal, bronchial, or conjunctival allergen challenges (NAC, BAC, and CAC, respectively) are very useful tools to evaluate the capacity of aeroallergens to induce organ-specific symptoms, especially when the clinical history is not sufficiently clarifying.[5]

The interest in identifying the allergic triggers of conjunctivitis, rhinitis, and asthma relies on the possibility of implementing specific preventive and therapeutic strategies.[6] Interventions to decrease the allergen burden can sometimes control respiratory symptoms (e.g., alpine altitude climate treatment).[7] Moreover, the recognition of the allergic triggers of these conditions can be paramount to decide on patient's life choices (place of residence, hobbies, pet keeping habit, etc.).[8] Finally, some therapeutic interventions (e.g., allergen immunotherapy (AIT) or anti-IgE treatment) are recommended by the clinical guidelines only for allergic individuals.[9–12] Nevertheless, the success of these interventions largely depends on the correct identification of the allergic triggers affecting each patient,[13] and organ-specific allergen challenges may be used for this purpose.[14]

The aim of this review is to summarize novel data regarding the methodology of NAC, CAC, and BAC, and to address these aspects in the context of existing clinical protocols. Moreover, the potential of organ-specific allergen challenges for precision medicine in respiratory conditions will be emphasized. In this regard, despite the lack of studies comparing AIT outcomes in patients diagnosed with or without organ-specific allergen challenges, it is reasonable to believe that these tests will favour better health outcomes in patients with airway diseases. Because allergen challenges are widely implemented in occupational medicine, this article will focus on areas of clinical medicine where their use has been scarcer.[15] Similarly, other types of specific challenges (e.g., lysine-acetylsalicylate provocations) are beyond the scope of this article.

2 | THE CLINICAL HETEROGENEITY OF AIRWAY ALLERGY

The results of atopy tests (skin prick test (SPT) and serum allergen-specific (s)IgE) differ among the phenotypes of airway allergy.[4] Atopic respiratory allergy (ARA) is characterized by the positivity of atopy tests, and the onset of symptoms upon exposure to the sensitizing allergen (atopic allergic individual).[8] A good correlation between the allergen sensitizations and the clinical response is required because atopic sensitization can be also asymptomatic.[16] Although the immunological basis for this phenomenon is poorly understood, it is common to find both atopic healthy individuals and atopic rhinitis or asthma patients who do not develop symptoms upon exposure to the allergens they are sensitized to (atopic non-allergic individuals).[16] Therefore, the investigation of the clinical relevance of the sensitizations detected is an essential step in ARA diagnosis. On the other hand, in some non-atopic rhinitis and asthma patients, allergen-specific nasal and/or bronchial reactivity can be demonstrated by NAC and/or BAC (non-atopic allergic individuals).[17] This disease phenotype has been termed local respiratory allergy (LRA). LRA has been identified in patients of all ages and racial background, although a lower prevalence in Asian populations might exist.[18] The majority of studies conducting a NAC in non-atopic patients with rhinitis reported a consistent prevalence of local allergic rhinitis, ranging 8%–84% in adults[19–22] and 4%–67% in children.[23–26] On the other hand, one study performing nasal provocation with house dust mite (HDM) in 19 German non-atopic young adults found no positive response.[27] Importantly, a systematic review published in 2017 concluded that the prevalence of local allergic rhinitis among non-atopic individuals with chronic rhinitis was 25% for adults and 16% for children.[28] Bronchial allergen-specific reactivity has been also reported in non-atopic asthmatics with local allergic rhinitis (local allergic asthma).[17,29] Interestingly, both ARA to a certain allergen and LRA to another allergen can coexist in the same individual, and the term dual respiratory allergy (DRA) has been proposed for this clinical scenario.[24,30] Similarly, allergic and non-allergic mechanisms can coexist in the same rhinitis patient, thus defining the mixed rhinitis phenotype.[31]

Another defining feature of airway allergy is the good response to AIT.[32] The capacity of this treatment to control symptoms, decrease the need for standard medication, improve the quality of life, and increase the allergen dose tolerated in organ-specific provocations has been demonstrated for both ARA[9,12] and LRA phenotypes.[29,33–37] Moreover, AIT is considered a disease-modifying therapy for ARA.[38,39] Of note, it is well established that AIT prevents aggravation in these patients, and displays a sustained benefit after discontinuation, as long as a ≥3-year cycle is administered.[40] On the other hand, the long-term effect of AIT in LRA patients, and the overall effect of AIT in DRA individuals remain to be investigated.

In summary, organ-specific allergen challenges are required to discriminate between allergic and non-allergic phenotypes, as well as, between different allergic phenotypes of chronic rhinitis, conjunctivitis, and asthma. Figure 1 shows the distribution of allergic phenotypes within the bigger picture of the distinct endophenotypes driving airway diseases.

3 | GENERAL ASPECTS OF ORGAN-SPECIFIC ALLERGEN CHALLENGES

Organ-specific allergen challenges are meant to reproduce the response of the airway mucosa to an aeroallergen in a controlled manner.[5] These tests are the gold standard for the identification of the allergic triggers of rhinitis, conjunctivitis, and asthma in the clinic.[41]

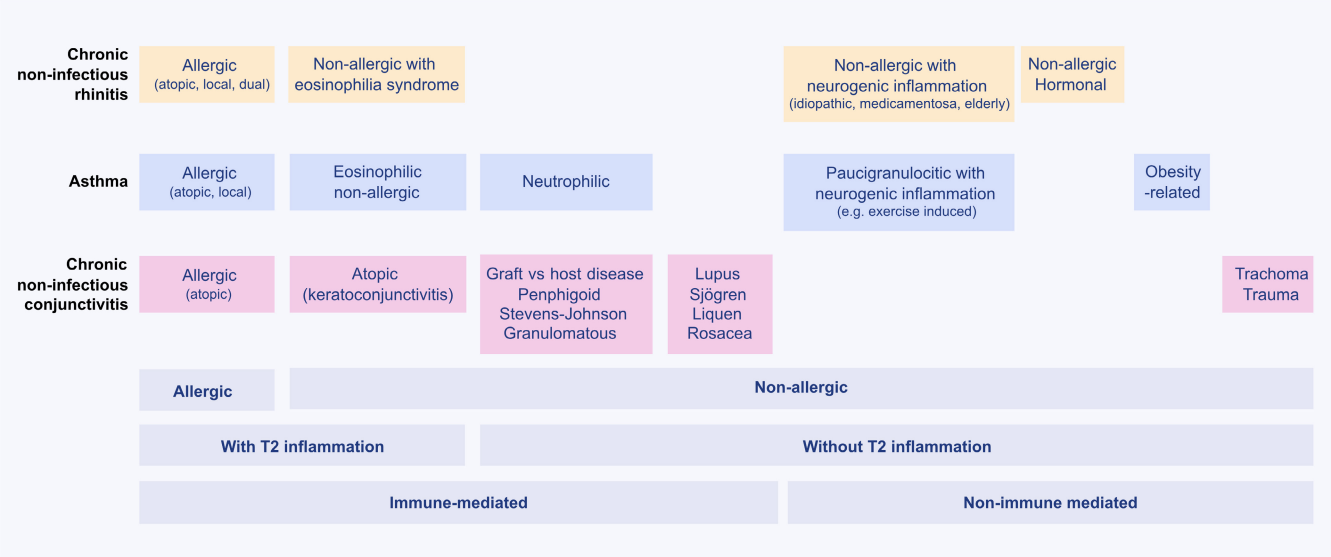

FIGURE 1 Endophenotypes of chronic non-infectious rhinitis, asthma, and chronic non-infectious conjunctivitis. Endophenotypes in the same column share common pathological mechanisms. Chronic rhinitis is usually divided into the allergic and non-allergic phenotypes. Asthma is usually divided into the T2 and non-T2 phenotypes. Chronic conjunctivitis is usually divided into the immune-mediated and non-immune-mediated phenotypes. Nevertheless, either dichotomy can be also applied to the diseases affecting the other mucosae (nasal, bronchial, or conjunctival).

As such, they are believed to display an optimal diagnostic accuracy and are used as standard comparators to calculate the sensitivity and specificity of other tests.[42]

3.1 | Indications of organ-specific allergen challenges

The BAC, NAC, and CAC are the gold standard for the identification of bronchial, nasal, and conjunctival allergen-specific reactivity, respectively.[43–45] Thus, these procedures can help phenotype atopic patients with discrepancies between the results of SPT/serum sIgE and the symptom pattern as assessed by the clinical history.[16,46] This clarification would be particularly useful in patients on standard pharmacotherapy who continue having bronchial, nasal, or conjunctival symptoms, as it can guide a different therapeutic approach (e.g., AIT and allergen avoidance measures) to achieve and maintain control.[47] Similarly, these tests may help decide the composition of AIT, especially in polysensitized patients with perennial symptoms.[48] Moreover, the CAC has been proposed to monitor the effect of AIT (e.g., to decide whether the treatment should be stopped after 3 years or continued up to 5 years),[49] although the NAC might represent an alternative in this regard. Moreover, the CAC can help differentiate between allergen-driven and intrinsic symptoms in patients with atopic keratoconjunctivitis.[50]

On the other hand, the NAC is needed to differentiate local allergic and non-allergic rhinitis in non-atopic individuals, and dual allergic rhinitis and mixed rhinitis in atopic subjects.[41] Moreover, the BAC would be required to identify bronchial allergen-specific reactivity in non-atopic asthmatics with a clinical history suggestive of allergy (local allergic asthma).[51] Conversely, direct (e.g., methacholine provocation) or other type of indirect (e.g., mannitol provocation) challenges are mostly used to detect bronchial hyperresponsiveness as an essential step in asthma diagnostic process.[52,53] Thus, the BAC and other bronchial challenges are complementary tests. Unlike the nasal and bronchial counterparts, the existence of allergen-specific conjunctival reactivity in non-atopic individuals ("local allergic conjunctivitis") has not been reported.[54]

In recent years, various works have investigated the utility of the NAC and CAC for the diagnosis of IgE-mediated food allergy (especially with plant foods sharing panallergens with pollens), due to their potential greater safety than oral food challenges.[55–57] The CAC has been also proposed for the diagnosis of latex allergy under the same rationale.[58] Nevertheless, these uses still require further investigation. Importantly, organ-specific allergen challenges can monitor the effect of AIT and other anti-inflammatory drugs in clinical trials,[59–62] or investigate the mechanisms of airway allergy.[63,64] To this end, they can be combined with the collection of different samples (e.g., lavage, brushing, scraping, biopsy, cytology, tears, sputum, etc.) at distinct time points.

3.2 | Contraindications of organ-specific allergen challenges

Shared temporary contraindications include the insufficient washout period for alcohol, tobacco, and any relevant drug.[5] The tests cannot be performed in pregnant women or during the 4 weeks following a respiratory infection, anaphylaxis, or surgery involving the airways.[65] A 6-month interval is required between a surgical

procedure in the ocular surface and a CAC. As a general rule, a good asthma control should be achieved before conducting a BAC[66] or NAC.[67] Moreover, these tests cannot be performed in patients with FEV1 <70%,[53] whereas more flexibility for asthma control and FEV1 values exist for the CAC.[65] On the other hand, the CAC cannot be conducted in patients with blepharoconjunctivitis, sicca syndrome, urban eye syndrome, or giant papillary conjunctivitis.[45] Although the NAC and CAC require minimal collaboration, they are hard to implement in preschool children.[65] Additionally, the ability to perform good spirometry maneuvres is a prerequisite for a BAC,[66] yet impulse oscillometry might represent an alternative for monitoring.

The lack of standardized allergen extracts represents a shared relative contraindication.[68] The inhaled or nasal routes are not associated with systemic allergic reactions, but aeroallergens can exceptionally trigger anaphylaxis through the oral route (e.g., ingestion of HDM-contaminated flour).[69] This scenario might constitute another relative contraindication.

Severe unstable oncologic, cardiopulmonary, autoimmune, or endocrine conditions represent shared absolute contraindication for organ-specific allergen challenges.[5]

Table 1 summarizes the indications and contraindications of the NAC, BAC, and CAC.

4 | METHODOLOGY OF ORGAN-SPECIFIC ALLERGEN CHALLENGES

4.1 | Common aspects

Provocations with seasonal allergens should be performed out of the pollen season (4-week interval), whereas challenges with perennial allergens should be conducted at the time of the minimal allergen burden.[65,70] These procedures require standardized allergen extracts which are neither cheap nor easy to purchase.[68] In addition, extracts for CAC lack any preservative to avoid an irritant effect over the conjunctiva, and must be used the same day they are opened. It is crucial to check that the appropriate washout period of every potential confounder (including over the counter medications) has been kept by the patient (Table 2), that all necessary equipment to treat an anaphylactic reaction or a bronchospasm is readily available, and that the informed consent has been provided.[5] Asthma control needs to be assessed before starting the procedure.[66] The room where the test is performed should be quiet and with stable temperature (20±1.5°C) and humidity (40%–60%).[65] Importantly the patient should acclimate to the room conditions for 15 (NAC and CAC) to 30 (BAC) minutes before the test is started.[5]

4.2 | Organ-specific aspects

4.2.1 | Nasal allergen challenge

The European Academy of Allergy and Clinical Immunology (EAACI) published a standardized methodology for the NAC in 2018.[44] Patients should undergo a nasal endoscopy before being subjected to the provocation. This step is required to ensure sufficient permeability of the nostrils, and to rule out inflammatory conditions other than rhinitis and major anatomical abnormalities.[67] In any case, the examination should not be performed the same day the NAC is conducted. The monitoring of the NAC should always include a subjective (symptoms score such as Lebel or Linder scores, or Visual Analogue Scale) and objective (e.g., acoustic rhinometry, peak nasal inspiratory flow, active anterior rhinomanometry, etc.) parameter.[44] The NAC is started by a baseline measurement of both parameters to check that the patient is asymptomatic or suffers only from mild disease.[65] Subsequently, a control challenge (e.g., the diluent of the allergen extract) should be performed. If nasal hyperreactivity is excluded (second measurement), allergen administration can be initiated.[5] EAACI accepts several methods for intranasal allergen application, but micropipette and nasal spray are the two most widely utilized.[44] Bilateral application is recommended to control for the nasal cycle. A third monitoring should be conducted 10–15 min after allergen administration. According to EAACI, the NAC should be considered positive if moderate changes occur simultaneously in both objective and subjective parameters, or if clear changes are seen in at least one parameter.[44] Nevertheless, the cut-off points for most NAC-monitoring methods have not been clearly established.[71] On the other hand, a recent article identified that a decrease ≥25% in volume 2–6 cm of acoustic rhinometry (the area corresponding to the head of the lower turbinate in adults) has an excellent accuracy for the identification of allergen-specific nasal reactivity, and that the addition of a symptom score does not improve the diagnostic performance of the test.[41] For diagnostic purposes a single allergen dose is usually enough. Conversely, a challenge with incremental doses can be considered when assessing the effect of an intervention (e.g., AIT), especially in research settings.[67] Although EAACI recommends to administer a single allergen per session, a validated protocol for NAC with up to four different allergens per session is also available.[72] This approach is especially useful to screen for local allergic rhinitis patients among the population of non-atopic individuals with chronic rhinitis. Positive reactions in the NAC rarely require treatment, and the patient can be safely discharged 60 min after the positive response.[44] Isolated late reactions are very uncommon, and they should not be taken as indicative of positivity.

4.2.2 | Bronchial allergen challenge

Unlike the NAC and CAC, classical BAC protocols are tailored to research needs and are hardly applicable in the clinic due to both safety and cost-efficiency matters.[73] A positive BAC is determined by the occurrence of an early asthmatic response (EAR) within the 3 h following allergen inhalation.[74] Some patients testing positive also experience a late asthmatic response (LAR) that can persist longer than 7 h.[70] Indeed, allergen-induced bronchoconstriction can be more profound than that triggered by mannitol or methacholine.[53] Thus, the BAC requires a long observation period in the clinic, sometimes including overnight stay.[66] Importantly, the LAR is the

TABLE 1 Comparative indications/contraindications of the nasal, bronchial, and conjunctival allergen challenges.

	Nasal allergen challenge	Bronchial allergen challenge	Conjunctival allergen challenge
Indications	Diagnosis of allergic rhinitis in patients with a discrepancy between symptoms and atopy tests Diagnosis of local allergic rhinitis Diagnosis of dual allergic rhinitis Diagnosis of occupational allergic rhinitis	Diagnosis of allergic asthma in patients with a discrepancy between symptoms and atopy tests Diagnosis of local allergic asthma Diagnosis of occupational allergic asthma	Diagnosis of allergic conjunctivitis in patients with a discrepancy between symptoms and atopy tests Diagnosis of occupational allergic conjunctivitis
	Selection of allergen immunotherapy composition in polysensitized patients with rhinitis	Selection of allergen immunotherapy composition in polysensitized patients with asthma	Selection of allergen immunotherapy composition in polysensitized patients with conjunctivitis
	Monitoring the effect of AIT in patients with allergic phenotypes of rhinitis		Monitoring the effect of AIT in patients with allergic phenotypes of rhinitis or conjunctivitis
			Identification of the allergic triggers in patients with atopic keratoconjunctivitis
Contraindications	Acute inflammation of the nose or paranasal sinuses Recent nasal surgery (4 weeks)		Non IgE-mediated disorders of the ocular surface Recent ocular surgery (6 months)
	Severe uncontrolled asthma or chronic obstructive pulmonary disease (e.g., FEV1 <50%) (might be temporary)	Uncontrolled asthma or chronic obstructive pulmonary disease (e.g., FEV1 <70%). Especially patients who worsen control or cannot keep a FEV1 ≥70% during the minimal washout period for antiasthma drugs (might be temporary)	
		Patients who cannot discontinue temporarily the intake of non-selective β blockers	
		Patients who cannot perform reproducible spirometry maneuvers (impulse oscillometry might be an alternative)	
			Use of contact lenses (temporary contraindication for 7 days)

Note: Severe unstable systemic diseases (e.g., oncologic, autoimmune, cardiologic, respiratory, endocrine). Pregnancy, preschool children, 4 weeks following an anaphylactic reaction, lack of standardized allergen extract.

most useful parameter for clinical studies (e.g., to assess the effect of a new anti-asthma drug in a clinical trial).[75–77] Of note, inhaled corticosteroids (ICS) greatly affect the LAR, whereas their effect over the EAR roughly disappears 12 h after administration on average.[78] Therefore, when the BAC is performed with research purposes a long washout period for ICS (e.g., 4 weeks) is required. Of note, partially controlled or moderate-to-severe patients can hardly tolerate this period. This fact impairs the correct identification of the allergic triggers of asthma in the heterogeneous population of asthmatics in the clinic. Nevertheless, partially-controlled individuals can still benefit from specific interventions for allergic asthma (e.g., HDM immunotherapy).[9,12] In order to progress in the clinical applicability of the BAC, a recent EAACI position paper has proposed modifications for the use of the test with diagnostic purposes.[43] These include shortening the washout period for ICS (from 4 weeks to 24 h) or to implement an active treatment (e.g., a dose of oral corticosteroids (OCS)) once the EAR is detected. Thus, this modified protocol has the potential to display a better safety and tolerability profile than the research BAC, as the LAR will become less frequent and/or severe. Of note, these modifications might help conduct the test in asthmatics who can only achieve partial control, thus guiding therapeutic interventions also in this population.

After the preliminary considerations (see Section 4.1), the EAACI position paper recommends to perform a baseline spirometry and FeNO measurement,[43] followed by a diluent inhalation (same diluent as for the allergen). If the patient does not experience bronchial hyperresponsiveness, allergen inhalation is started.[66] Unlike the NAC, the administration of progressively increasing doses (until reaching the target dose) is advised for the BAC.[70] Ten standardized biological units of allergen can be administered as starting dose in mild asthmatics.[79] For additional safety guarantees, the first dose can be decided by SPT end-point titration. Ten minutes after allergen inhalation the spirometry is repeated in duplicate. If there is a drop in FEV1 ≥20% (EAR), the BAC is considered positive and

TABLE 2 Comparative washout periods for different drugs before the nasal, bronchial, and conjunctival allergen challenges.

	Nasal allergen challenge	Bronchial allergen challenge	Conjunctival allergen challenge
Topical medication			
Topical antihistamines	4–5 days		2–3 days
Topical mast cell stabilizers	2–3 weeks		2–3 days
Topical corticosteroids	2–3 days		2–3 days–4 weeks
Cyclosporine (eye drops)	7 days		7 days–1 month
NSAIDs (eye drops)	7 days		7 days
Inhaled medication			
Short-acting β2-agonists and anticholinergics		8 h	
Long-acting β2-agonists and anticholinergics		72 h	
Corticosteroids		24 h–4 weeks	
Cromones		24 h	
Systemic medication			
Corticosteroids	2–3 weeks	8 weeks	2–4 weeks
Antihistamines	7 days	7 days	1–3 weeks
Leukotriene receptor antagonists	3 weeks	8 weeks	3 weeks
Metylxanthines		24 h	
Anti-IgE monoclonal antibody		6 months	
Anti-IL-5, anti-IL-5R and anti IL-4/13R monoclonal antibodies		6 months	
NSAIDs	1 week	1 week	
Tricyclic antidepressants	2–3 weeks	2–3 weeks	

Abbreviations: IL-4/13R, receptor for IL-4 and IL-13; IL-5R, receptor for IL-5; NSAIDs, non-steroidal-antiinflammatories.

subsequently stopped.[43] If this scenario does not occur, the next higher allergen dose is administered (commonly five consecutively higher doses are given if the patient does not react).[79] If a drop in FEV1 ≥20% is observed at any point before the 3 h following the inhalation of the highest allergen dose, the test is considered positive. In this case, EAACI protocol recommends to administer the patient a single dose of OCS (30 mg prednisone or equivalent), in addition to inhaled therapy.[43] Although the administration of a single OCS dose might increase the safety and facilitate the clinical implementation of the test, this recommendation needs to be validated in future studies. If the test is negative or 7 h after the recovery from a positive result the patient can be discharged,[66] but should be advised to re-start the controller anti-asthma medication, and to continue with home observation and regular monitoring of peak expiratory flow. Both inhaled medication and OCS should be self-administered by the patient if required. In case of borderline result (e.g., drop in FEV1 15%–20% during the 3 h following allergen inhalation) in a patient with shorter than optimal (<4 weeks->24 h) washout period for ICS, the interpretation of the BAC can be assisted by FeNO measurement 24 h after allergen inhalation.[43] In this regard, an increase ≥17.5 ppb shows a good correlation with a ≥20% drop in FEV1.[80] Importantly, the BAC only permits to test one allergen per session. Isolated late reactions are rare if the patient receives the target allergen dose.[70] In any case, they should not be taken as indicative of positivity.

4.2.3 | Conjunctival allergen challenge

A standardized methodology for CAC was published by EAACI in 2017.[45] After assessing for the preliminary considerations, the ocular surface should be examined to rule out significant conjunctival inflammation. Current protocols for CAC are based on that developed by Abelson and Loeffler.[81] The procedure includes the instillation of four incremental allergen doses in the inferior-external quadrant of the bulbar conjunctiva. The allergen is administered using 20–40 μL eye drops.[45] The contralateral eye serves as negative control and is instilled with physiological serum. Positivity is assessed 15 min after each instillation on the basis of the total ocular symptom score in which itching is scored from 0 to 4 and tearing, redness, and chemosis are each scored from 0 to 3.[82] A test is considered positive when the cumulative score reaches at least five points. In recent years, several methods to assess the response in a more objective manner (photographic scales of hyperemia, digital images, and confocal microscopy) have been proposed,[83] but they have not reached yet the clinical practice. An observation period of 2 h at the hospital is recommended after a positive CAC. Although rhinitis may occur following a CAC, the symptoms elicited by a positive test are usually controlled by eye topical antihistamines.[49] Patients who do not react to the highest allergen dose can be discharged but should follow home monitoring for 24 h. Unlike the NAC and BAC, isolated late reactions are

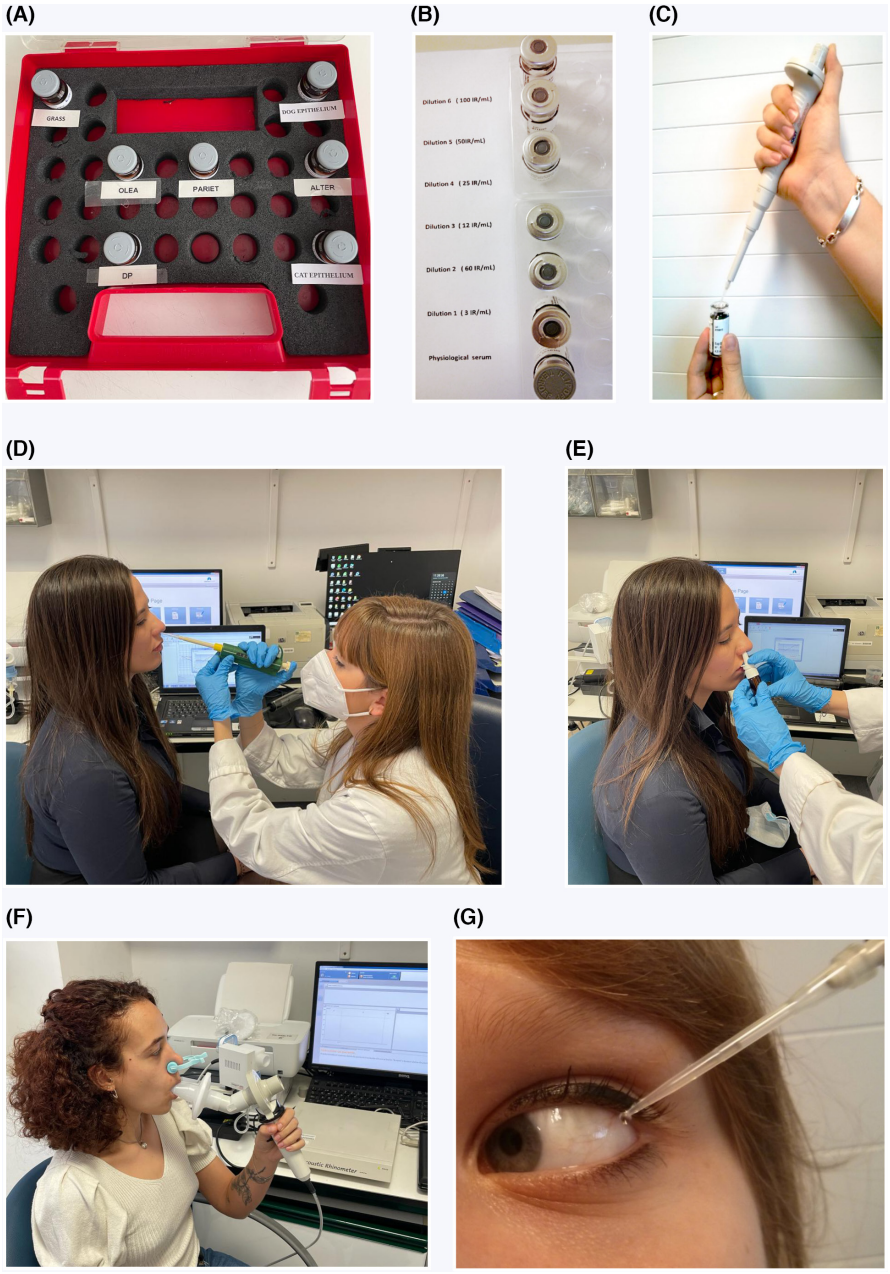

FIGURE 2 Allergen administration in organ-specific challenges. (A) Undiluted allergens for nasal provocation. The same extracts after dilution can be used for bronchial provocation. (B) Diluted extracts (incremental concentrations) for conjunctival provocation. (C) Pipetting of allergen extract for nasal or conjunctival provocation. (D) Allergen application by micropipette during a nasal provocation. (E) Allergen application by nasal spray during a nasal provocation. (F) Allergen inhalation using a dosimeter during a bronchial provocation. (G) Allergen instillation by micropipette during a conjunctival provocation.

considered indicative of positivity in the CAC, according to EAACI position paper.[45] To test a single allergen per session is recommended in the CAC.

Figures 2 and 3 show the methods for allergen administration and monitoring, respectively, used in organ-specific allergen challenges.

5 | SAFETY

A study analysing data from 11,499 NACs conducted in 6348 children and adults (including 510 asthmatics) investigated the safety of the test.[84] The NAC was well tolerated in 99.97% of cases, with only four procedures inducing symptoms outside the nasal and conjunctival mucosae. Of note, these reactions were restricted to the throat and responded to oral antihistamines. Similarly, the vast majority of positive NACs required no treatment or were controlled with oral antihistamines. Interestingly, the application of the allergen through either micropipette or nasal spray, and the administration of either one, or two-to-four allergens per session were equally safe.

There is also extensive data on the safety of the BAC when performed in mild asthmatics with unequivocal allergic phenotype.[75–77] In this population, inhaled salbutamol is commonly sufficient to treat the positive reaction, and to recover the baseline FEV1 value.[85] Nevertheless, the safety of the BAC needs to be investigated in the heterogeneous population of asthmatics in the clinic. Importantly, safety has been classically considered a limitation for the clinical implementation of standard BAC protocols.[73] Given the risk of losing

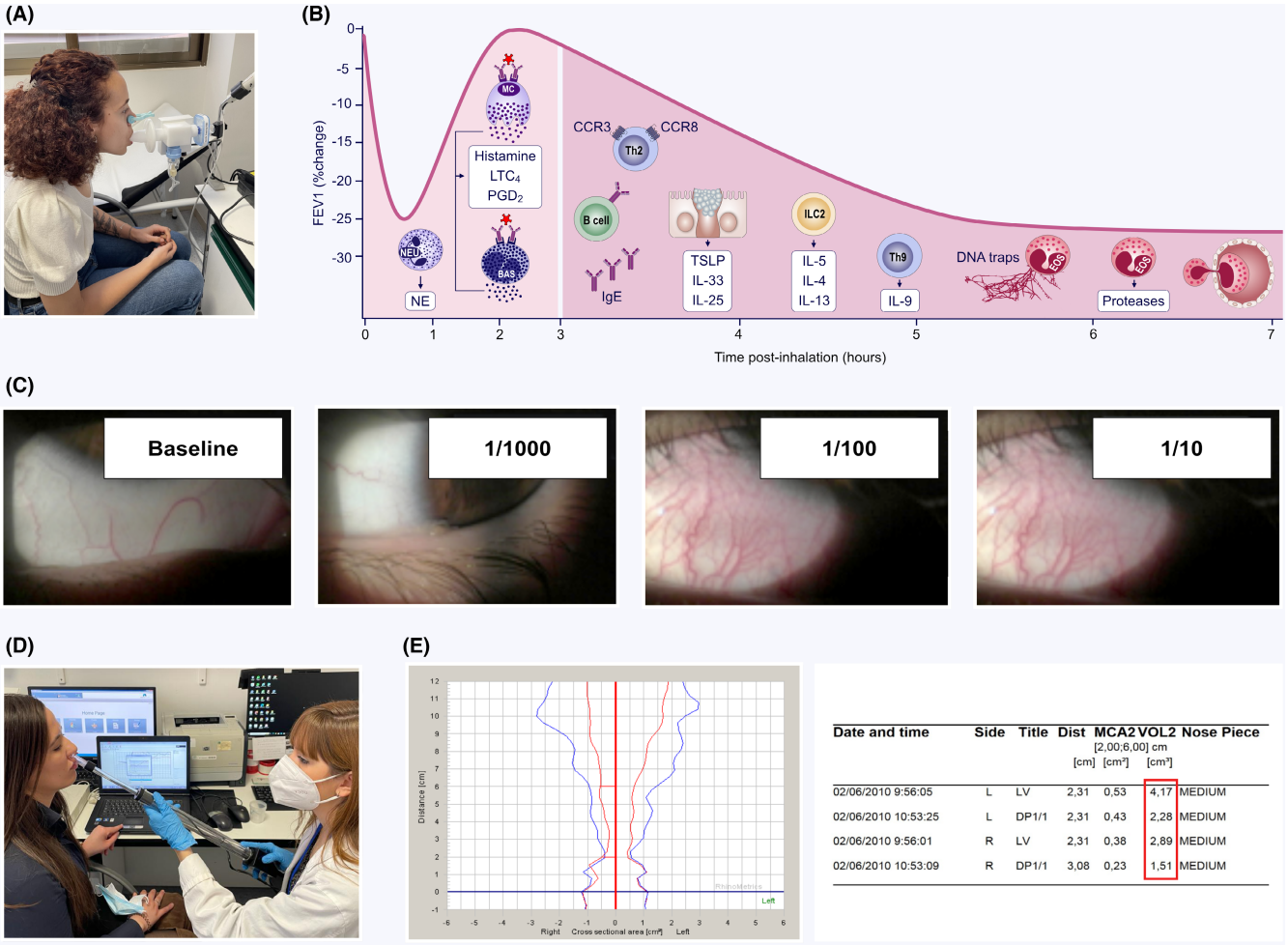

FIGURE 3 Monitoring of organ-specific allergen challenges. (A) Monitoring of a bronchial provocation by forced spirometry. (B) Typical two-phase reaction in a positive bronchial allergen challenge. The graphic depicts the changes in FEV1 during the early and late asthmatic responses, together with the cells involved in the responses. Reproduced with permission of the authors from Agache et al.[43] (C) Positive response in a conjunctival provocation with the highest allergen dose. (D) Monitoring of a nasal provocation by acoustic rhinometry. (E) Positive response in a nasal provocation. The upper part depicts a graphic with intranasal volumes (the blue and red lines represent the pre-challenge and post-challenge measurements, respectively). The two short red lines perpendicular to the volume lines mark the area located 2–6 cm from the nostril entrance. The lower part represents the volumes obtained during the measurements.

asthma control after a positive BAC, the centers conducting this test should count on high standards for patient follow-up and availability of medical assistance after discharge.[43]

Similarly, there are no specific studies on CAC safety, although the test is generally considered a safe procedure even in asthmatic patients.[86]

6 | REPRODUCIBILITY

A recent work conducted three consecutive NACs with the same allergen in 710 subjects including healthy individuals, and patients with distinct rhinitis phenotypes.[84] The reproducibility and negative and positive predictive values were 97.32%, 100%, and 92.91%, respectively. Of note, no false positive result was observed. Many studies have also demonstrated the reproducibility of the BAC in mild allergic asthmatics.[87] Importantly, the intraclass correlation coefficient for the allergen dose eliciting the EAR, the LAR, and a significant increase in sputum eosinophilia are .80, .77, and .60, respectively (all values within the good-to-excellent range).[88–90] On the other hand, no published study has investigated the reproducibility of the CAC, although a 92% concordance for HDM has been reported in a congress communication.[82] To ensure reproducibility, 1-month interval is recommended between two consecutive BACs with the same or different allergen.[88] Similarly, 1-week interval is required between two consecutive NACs or CACs with the same or different allergen, or between allergen challenges targeting different organs.[91] Table 3 summarizes the main aspects about the safety and reproducibility of NAC, CAC, and BAC.

TABLE 3 Comparative safety and reproducibility of the nasal, bronchial, and conjunctival allergen challenges.

	Nasal allergen challenge	Bronchial allergen challenge	Conjunctival allergen challenge
Safety	When the allergen is administered by aerosol spray or micropipette, the test is an extremely safe technique, even in asthmatic patients Non-troublesome extra-nasal upper respiratory symptoms/signs (oral, otic, or lingual pruritus/swelling, shore throat) might occur	Profound and treatment-resistant bronchoconstriction might occur, especially during the late asthmatic response. Peripheral venous access can be taken for additional safety	Extremely safe technique with symptoms restricted to periorbital area (orbital edema or conjunctival chemosis) or to the nostril
	Late reactions are rare A 1-h observation period at the hospital is recommended after a positive test	15%–90% of patients experience late reactions (the proportion is allergen-specific). A 7-h observation period at the hospital is recommended after a positive test	Late reactions can occur A 2-h observation period at the hospital is recommended after a positive test
Reproducibility	ICC for acoustic rhinometry: .66–.89 Concordance (%) for nasal spray technique: 96.24 and for micropipette technique 97.79 The presence of asthma does not alter the reproducibility of the nasal allergen challenge	ICC for EAR: .80 ICC for LAR: .60–.77	Concordance (%) for house dust mite and grass pollen 92. Not measured for other allergens
	Recommended minimal interval between consecutive NAC with the same or different allergen: 1 week Recommended minimal interval between NAC and BAC or CAC with the same or different allergen: 1 week	Recommended minimal interval between consecutive BAC with the same or different allergen: 4 weeks Recommended minimal interval between BAC and indirect bronchial challenge: 3 weeks and between BAC and direct bronchial challenge: 2 weeks	Recommended minimal interval between consecutive CAC with the same or different allergen: 1 week

Abbreviations: BAC, bronchial allergen challenge; CAC, conjunctival allergen challenge; EAR, early asthmatic response; ICC, interclass correlation coefficient; LAR, late asthmatic response; NAC, nasal allergen challenge.

7 | FUTURE DIRECTIONS

7.1 | Diagnostic implications of the united airways concept

Although airway allergy can present with distinct organ-specific manifestations (rhinitis, conjunctivitis, or asthma), the risk factors, triggers, and immunopathological mechanisms affecting the different organs are highly overlapping.[92] Similarly, therapeutic or preventive interventions have the potential to improve symptoms in both the upper and lower airways.[93,94] This phenomenon is usually summarized by the expression "united airways". This concept was initially applied to the naso-sinusal and bronchial mucosae, but it is now clear that other organs (conjunctiva, oral mucosa, lung parenchyma, etc.) can also be pathologically affected in patients with airway allergy.[95–99] Therefore, the clinical expression of the disease relies largely on the inflammatory interplay among the different boundaries exposed to aeroallergens.[100] In this regard, the naso-sinusal mucosa is connected through bidirectional neural reflexes with both the conjunctival and bronchial mucosae.[101] Moreover, the inflammatory mediators released to the airway lumen might travel to distant areas of the respiratory mucosa.[102] Of note, the NAC in allergic rhinitis patients without asthma induces a drop in the nasal and bronchial volumes together with an inflammatory infiltrate detectable in

BOX 1 Future research perspectives.

1. Investigation of the immunological basis regulating the clinical expression of atopic sensitization.
2. Further characterization of the local and dual respiratory allergy phenotypes.
3. Investigation of the safety and reproducibility of the conjunctival allergen challenge.
4. Investigation of the diagnostic performance and safety of the bronchial allergen challenge in patients other than mild asthmatics.
5. Validation of a protocol for bronchial allergen challenge applicable in the clinical practice.
6. Investigation of the capacity of the nasal and conjunctival allergen challenges to phenotype the disease affecting the airways regardless of their organ-specific manifestations.
7. Investigation of the capacity of the basophil activation test to diagnose the local and dual respiratory allergy phenotypes in individuals other than rhinitis patients.
8. Investigation of the capacity of the basophil activation test to discriminate between allergic and non-allergic individuals among the population of atopic patients.

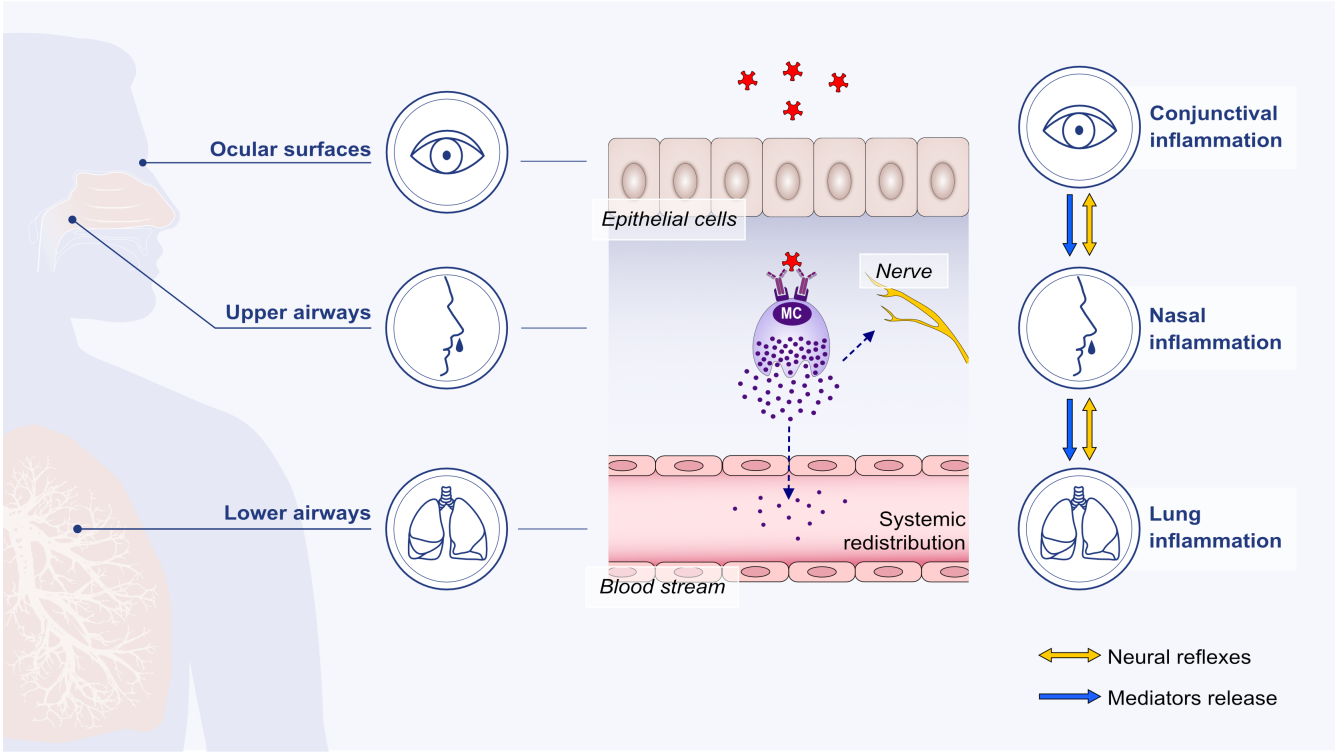

FIGURE 4 Mechanisms of reciprocal affection in the united airways. Conjunctival inflammation (e.g., allergen-driven) can trigger the naso-ocular reflex and induce nasal inflammation (e.g., activation of mucosal glands). Similarly, nasal inflammation can stimulate neural reflexes to mediate bronchial changes (e.g., bronchoconstriction). These reflexes can also function bidirectionally. Inflammation in the conjunctival, nasal, or bronchial mucosae releases mediators to the blood stream that can be redistributed and induce inflammation in distant mucosal organs. Moreover, inflammatory mediators released in the conjunctiva can travel through the nasolacrimal duct to trigger nasal symptoms. Similarly, mediators released to the airway lumen can induce inflammatory changes in lower segments of the airways.

the nasal and bronchoalveolar lavages.[103] Moreover, the conjunctival instillation of an allergen to a patient with allergic conjunctivitis triggers the activation of the naso-ocular reflex together with the release of inflammatory mediators, which can be transported to the nostril through the naso-lacrimal duct.[99] This interplay also occurs in the opposite direction, and either way induces nasal and conjunctival symptoms regardless of the organ where the allergen was given. Moreover, inflammatory mediators can be also released to the blood stream and be transported anywhere in the organism (Box 1).[104]

Interestingly, these immunopathological links (Figure 4) might have diagnostic implications. Because the CAC and NAC are safer than the BAC, and the CAC is considerably shorter than the NAC and the BAC, it might be interesting to explore the capacity of the CAC and NAC to identify the allergic triggers of airway diseases regardless of their organ-specific manifestations. Of note, a study conducting CAC and BAC in a small group of asthmatics sensitized to HDM showed a perfect correlation between the two tests.[105] On the other hand, the CAC is increasingly used as a surrogated biomarker of response to AIT in clinical trials with allergic rhinitis patients.[62] Similarly, the correlation between the NAC and BAC results in patients with rhinitis and asthma who are sensitized to HDM ranges 83%–89%.[106,107] Thus, several publications have recently proposed to integrate the NAC (in an earlier step than the BAC) in the diagnostic algorithm of HDM-driven allergic asthma, in order to identify patients who can benefit from AIT.[43,108] Although the correlation between the nasal and bronchial responses might differ among allergens,[109] it is important to note that international

BOX 2 Major milestones discoveries.

1. Demonstration of the safety of the nasal allergen challenges in the heterogeneous population of rhinitis patients in the clinic, and of the safety of bronchial allergen challenge in mild asthmatics.
2. Demonstration of the reproducibility of the nasal and bronchial allergen challenges.
3. Identification of cut-off points for the monitoring of the nasal, conjunctival, and bronchial allergen challenges.
4. Publication of standardized and clinically applicable protocols for nasal and conjunctival allergen challenges.
5. Publication of a proposed methodology for bronchial allergen challenge applicable in the clinical practice.
6. Demonstration of the utility of the basophil activation test for the diagnosis of local and dual allergic rhinitis.

TABLE 4 Comparative features of organ-specific and ambient allergen challenges.

	Organ-specific challenge	Ambient challenge
Advantages		
Principle	Standardized protocol representing the gold standard for the individual identification of the allergic triggers of rhinitis, conjunctivitis and asthma	Exposure of a group of patients to stable airborne allergen concentrations under defined conditions that mimic natural exposure
Indications	Clinical: identification of the allergic triggers of rhinitis, conjunctivitis and asthma in both atopic and non-atopic individuals. Monitoring the effect of allergen immunotherapy Research: investigating the effects of anti-allergic treatments and the mechanisms of airway allergy	Research: evaluation of clinical studies Phase I–III clinical trials (regulatory requirements). Dose finding. Onset of action
Methodology	Individual exposure Fast, easy, reproducible	Collective exposure to a sole allergen, flexible concentrations, reproducible
Allergens	Available aqueous extracts	Possibility of using allergenic raw materials or lyophilized extracts (good medical practice products)
Safety: secondary effects	Safe and reproducible method. Isolated late reactions are rare in nasal and conjunctival challenges	Safe method. Adapted designs to detect priming effects. Possible occurrence of isolated late reactions
Practice	Less expensive (per patient)	Applicable to large cohorts of patients
Pitfalls		
Practical aspects	Time- and resource-consuming nature	High cost
	Higher allergen doses than natural exposure	Longer and repeated exposures: the challenge might require a preliminary concentration-finding provocation
	No correlation with symptom magnitude or temporality compared to natural exposure ambient	Requires a specialized technical facility and highly qualified staff
	Reduced number of allergen extracts available. Allergen extracts do not reflect natural exposure in all cases	Need harmonization of chamber techniques (determination of batch-specific allergen profile and concentration of the allergenic material used) and standardized symptom scoring. Technical differences between existing facilities not allowing multi-center clinical studies

guidelines for asthma only recommend immunotherapy with HDM (Box 2).

7.2 | The role of ambient challenges

Ambient challenges are the method best mimicking natural exposure conditions to aeroallergens.[110] They constitute a regulatory requirement to obtain market authorization through phase 1–3 clinical trials, and are very useful in dose-finding (phase 2) and proof-of-concept trials[111] Moreover, they represent the only method to assess the onset of action and the effect size of new treatments for airway allergy.[112] Thus, they have been widely used to evaluate the performance of AIT and anti-inflammatory drugs in clinical studies.[113] In these procedures, several patients are exposed simultaneously to a stable and well-defined airborne allergen concentration which is closer to natural exposure than that of organ-specific allergen challenges.[114] Nevertheless, the few studies investigating the correlation between ambient challenges and NAC are hard to interpret, because the monitoring of the nasal response in the former was not conducted as per guidelines.[109] Despite their utility in research studies, it is highly unlikely that ambient challenges will ever reach the daily practice due to cost-efficiency matters. Table 4 shows a comparison between organ-specific and ambient allergen challenges.

7.3 | In vitro alternatives for organ-specific allergen challenges

Organ-specific allergen challenges are time-consuming procedures which need to be performed by trained personnel and require technical resources.[5] Therefore, it would be interesting to explore more patient-friendly alternatives (like in vitro biomarkers) able to recognize allergic patients among the heterogeneous population of atopic and non-atopic individuals in the clinic.

Some works tried identifying local allergic rhinitis patients by measuring sIgE in the nasal secretions, and yielded conflicting results

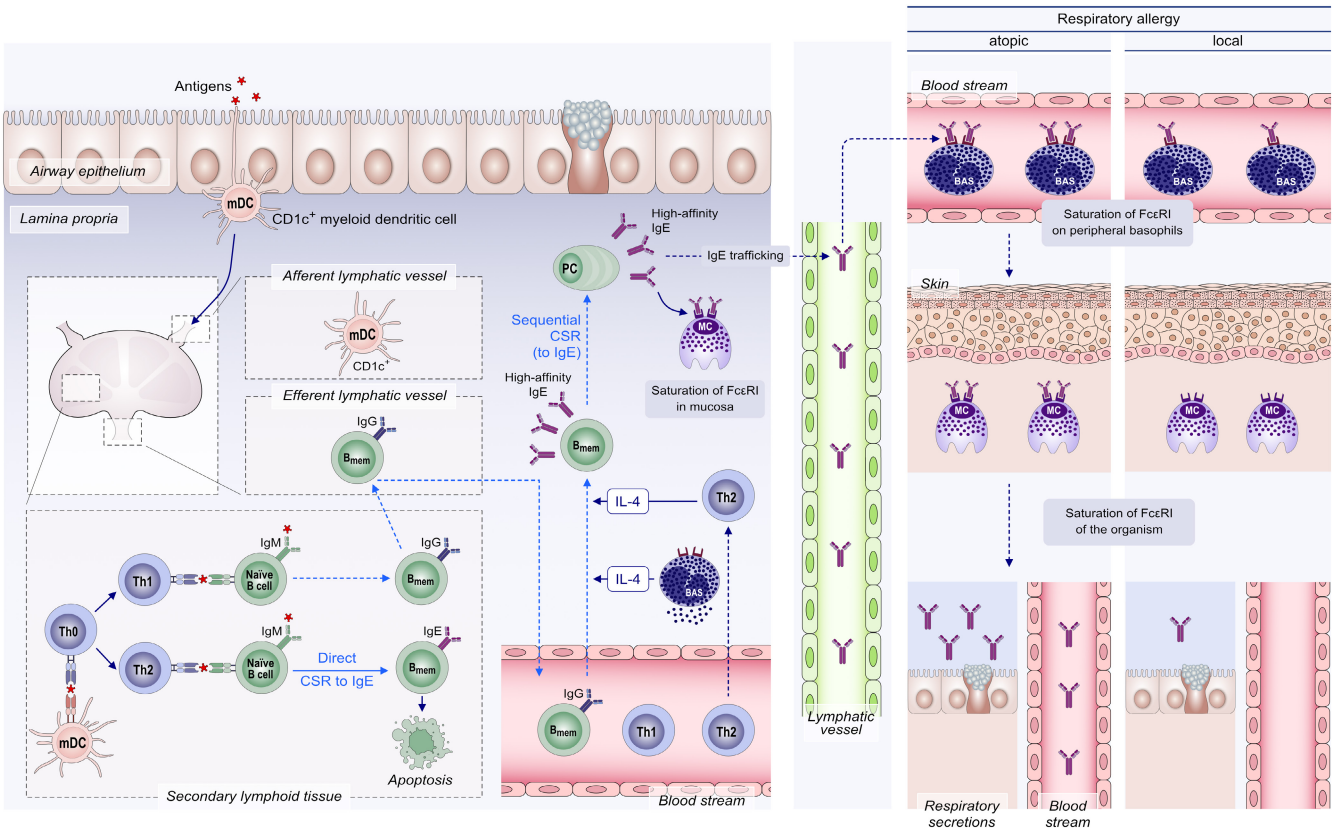

FIGURE 5 Mechanisms of airway allergy. During the sensitization phase of airway allergy allergen-specific (s)Th2 cells, Th1 cells and IgG1+ B cells are generated in the secondary lymphoid tissues (SLT) with subsequent migration to the blood stream. Conversely, IgE+ B cells do not complete their maturation and die by apoptosis in SLT. During the effector phase of airway allergy, the allergen stimulates the recruitment of sTh2 and sIgG1+ B cells to the airway mucosa and the release of IL-4. IL-4 mediates in turn the class switch recombination to IgE of IgG1+ B cells in the airway mucosa, giving rise to IgE+ plasma cells. sIgE released by plasma cells sensitizes first mucosal resident mast cells. After the saturation of FcεRI receptor system in the mucosa, sIgE traffics through the lymphoid vessels to the blood stream where it binds to FcεRI expressed on circulating basophils. After the saturation of peripheral basophils, sIgE is distributed to the organisms where it sensitizes resident mast cells, including those in the dermis. Only, after the saturation of the whole FcεRI receptor system in the organism, sIgE can be found free in serum or in other biological fluids like the respiratory secretions. This binding sequence of IgE to the receptors expressed in the different organs explains the status of the diagnostic biomarkers (organ-specific allergen challenges, basophil activation test, skin prick test, and serum sIgE) in the distinct phenotypes of airway allergy.

(0%–20%).[115,116] In any case, this data is hard to interpret since the proportion of patients who would have tested positive in a NAC is unknown. In this regard, it seems logical to focus the efforts for nasal sIgE identification on those individuals with proven nasal allergen-specific reactivity (positive NAC), as sensitization (either systemic or local) does not equal allergy.[16] Moreover, the collection of local samples poses tolerability issues and sometimes requires a previous allergen challenge to obtain acceptable sensitivity.[117] In addition, the measurement of local sIgE lacks of standardized methodology or validated cut-off points. Finally, the measurement of sIgE in respiratory secretions might not be the optimal method to identify LRA patients,[42] given that these individuals do not have detectable sIgE in serum, and both biological fluids are ultimately connected through the lymphoid vessels.[4]

On the other hand, the BAT has shown promising results for LRA identification, as >50% of individuals with local and dual allergic rhinitis display positive results with the allergen they react to in the provocation.[30,92,118–120] The BAC counts also on optimal specificity, standardized methodology, and validated cut-off points for positivity.[121,122]

A thorough elaboration of the mechanisms explaining why LRA/DRA patients display sIgE against aeroallergens attached to the membrane of peripheral basophils is beyond the scope of this review. In brief, recent data indicate that, similar to AR, in LRA individuals, sIgE against aeroallergens is produced at the mucosal level, following sequential class switch recombination of IgG_1+ B cells.[92] After sensitizing nasal resident effector cells, sIgE traffics to the bloodstream through the lymphoid vessels to sensitize circulating basophils in first place. The extremely high affinity of IgE for FcεRI (K_d: 10^{-10}) would explain why LRA patients can display membrane-bound sIgE in peripheral basophils without having free sIgE detectable in serum.[123] These mechanisms are explained with further detail in Appendix S1 and Figure 5.

In any case, the capacity of the BAT to phenotype the conjunctival or bronchial disease, or to discriminate between allergic and non-allergic atopic subjects remains to be investigated.

8 | CONCLUSIONS

For decades, the demonstration of atopy (as defined by SPT positivity and/or detectable serum sIgE against aeroallergens) has been regarded as a prerequisite for the diagnosis of airway allergy. Nevertheless, recent evidence indicates that atopy and allergy represent two different phenomena. Some allergic patients with rhinitis and asthma are not atopic (LRA phenotype), while healthy individuals or even rhinitis, conjunctivitis and asthma patients can display positive SPT results without experiencing symptoms upon exposure to the allergens they are sensitized to. Finally, allergy with and without concomitant atopy can coexist in the same patient (DRA phenotype).

Given the many limitations of atopy test to identify bona fide allergic individuals and the current lack of in vitro tests for allergy, it is crucial to progress in the clinical implementation of NAC, CAC, and BAC. Because the BAC is less safe and more time-consuming that the other tests, the capacity of the NAC and CAC to phenotype asthma patients is worth of investigation. In summary, the identification of the allergic triggers of rhinitis, conjunctivitis, and asthma through organ-specific allergen challenges constitutes an opportunity for a better selection of patients benefitting from interventions with disease-modifying potential.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to disclose in relation to this work.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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