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Allergy Diagnosis - an Application to Dog

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ABSTRACT: Better living conditions of people and their companion animals are possibly playing an important role in sensitization and allergy as emerging conditions, either in human or veterinary medicine. Animal consultation because of pathologies regarding allergy is increasing much beyond the common atopic dermatitis to flea allergens. Several sources of air-born allergens, as well as many food allergens are also frequent causes of allergic reactions, showing different target organs from skin to eye and respiratory or digestive systems. This growing stream in veterinary clinical pathology needs to run trough well established guide-lines, either in clinical or in complementary diagnosis field. This review manuscript aims to integrate the basal knowledge on sensitization etiopathogeny, concerning patient immune response and allergen nature and diversity, and diagnosis, starting with a standard and accurate diagnostic clinical protocol and continuing trough several possible laboratory ways to extend and clarify the diagnosis. Our goal is to contribute for the clinical-laboratorial diagnostic course improvement, attending to different complementary diagnostic methods already available for veterinary diagnosis and to others that may be very useful in the near future, in spite of their actual lack of standardization for veterinary use. Only a deep knowledge about the wide range of available diagnostic methods, with their specific capabilities, will give veterinary clinicians the necessary information about the appropriate choice for each diagnostic proposal, which will be further and further demanding.

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ETIOPATHOGENY

THE ALLERGENS

When a foreign material contacts a superior living being, an immunologic reactions cascade is triggered by molecular substances called antigens (1). At this contact several individuals develop a kind of response generically called sensitization, characterized by the synthesis of specific IgE. Those IgE-inducing biological or merely chemical substances are designated allergens (2). Those, which way of contact or sensitization is mainly inhalatory are called aeroallergens, while those contacting mainly by the digestive mucosa are called food allergens (1).

Aeroallergens are frequent triggers of respiratory tract reactions, while food allergens usually trigger reactions at the digestive tract (1). One described exception in humans is asthma after the ingestion of snails (3-12, among others). Exposure to garlic powder or to vapors form the boiling of crustacean an asthma after ingestion, or hives following the ingestion of honey from sunflower in pollen-sensitized patients are also classical examples (13). By analogy, inhalant allergic dermatitis is also a current condition in veterinary medicine (14).

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Skin contact allergens cause a cutaneous reaction characterized by blush and angioedema in acute situations or by eczema in chronic developments. Among the most severe allergic responses, systemic reaction to parenteral administration of drugs, such as penicillin, a classical cause of anaphylactic response, may be found.

WHAT MAKES AN ALLERGEN ALLERGENIC

Most major allergens (recognized by more than 50% of the sensitized patients) known are proteins within a molecular weight range from 3 to 80 kDa. Although, no chemical, structural or functional identity has been clearly identified that could explain the capacity to induce IgE response (15). Eventually, some enzymatic activity (16) or characteristics like thermo stability and resistance to acid denaturation and digestive proteolysis (17) may also stand as the basis of an improved capacity to induce an allergic response.

Twelve years passed over, the conclusions of Liebers et al. (1996) (15) persist valid in the concept that a structural chemical group could exist as a common denominator for the sensitization and possible triggering of the clinical reaction. In fact, the exact IgE response-trigger

antigenic structure is very difficult to identify, because individuals are exposed to a variable number of allergenic sources, like pollens (18) or mites (19).
Aalberse (2000) (20) referred that the potential for IgE-binding and associated clinical symptoms could be also related to physical properties, like three dimensional stability and size, and with immunological properties, like affinity and epitope valence. The observation of three dimensional models of the secondary and tertiary protein structure may, in fact, help to understand the affinity of the respective epitopes. From the analysis of 40 allergenic proteins, form which the three dimensional structure could be predicted by homology, 4 structural families were proposed. The grouped details form those allergens pointed to highly heterogeneous structures without any particular property that could act as incompatible to sensitization or allergy (20).

ALLERGENS MAY BE CROSS REACTING

An important point also coming out from those observations is that the structural similarity between proteins from different sources stands as the molecular basis of cross reactivity in allergy (18).
Aalberse et al. (21) referred that cross reaction between some allergens was possibly associated with the existence of IgE directed to carbohydrate cross-determinants (CCD), what per se may stand as a start for the detection of common structural aspects related with the induction of sensitization. Besides allergens themselves, IgE relevance for cross reaction seems to be influenced by several other factors, such as the immunologic response to the allergen and the level of exposure. Proteins with a sequential identity lower than 50% will seldom react in a cross fashion (20). For that it is frequently necessary more than 70% of sequential identity (22). A protective property, with a possible important role against cross-reaction between allergens and patient self homologous proteins is their level of similarity, since a close pattern between them could act as a protective factor against sensitization. Nevertheless, if a directed immunological reaction occurs, it may also lead to IgE-mediated auto reactivity (23, 24).
In fact, several examples of cross reacting allergens are available, mostly described towards human allergy, like the homologous of Bet v1 (allergen nr 1 form Betula verrucosa), or proteins produced in situations of plant stress for defense against pathogens (presents in apple, cherry, apricot, carrot, parsley, celery potato and hazelnut), where the primary sensitizing way in humans, is inhalatory and a common food allergy manifestation is the oral allergy syndrome (OAS) (17). Another example are the lipid transfer proteins (LTP), implicated in the production of plant cuticula and in defense. These are the principal implicated proteins in human allergy to rosaceae fruits, such as peach, apple, plum, apricot and cherry, in Mediterranean countries. These LPT present several characteristics, such as thermo stability (resisting to culinary treatment) and resistance to denaturation and digestive proteolysis. This runs in favor of a powerful allergenic effect at the digestive level, allowing them to reach the intestinal immune system with a preserved immunogenic conformation and induce sensitization. LPT are also present in numerous other plants, including pollens form birch, artemisia, plane tree and in latex. It is though that human cross sensitization to and between LPT is favored by the conservation of a high specie-to-specie homology (17).
Another example of allergens commonly associated with cross reactions in humans is profilin, a plant cytoskeleton protein, highly conserved from one specie to another. It is known that 10 to 20% of pollinic humans present cross sensitization between profilins and between carbohydrates (17).

FOOD ALLERGENS

Concerning food allergens, these are commonly glycoproteins with 15 to 50 kDa and, besides the molecular weight that may facilitate their absorption trough the digestive mucosa, a good immunogenic capacity depends on the number of epitopes of the antigenic molecule (13). The majority of food allergens are proteins with, at least, two IgE-recognized epitopes and the pattern of recognition by different individuals may vary with the replacement of a single aminoacid, which may change IgE recognition (25).
In food allergy, beyond the intrinsic characteristics of proteins, culinary treatment and digestibility are factors to be taken in account for the level of structural preservation and consequent availability of epitopes, while for aeroallergens, dimension and solubility should represent important characteristics (26, 27). Then, some food protein characteristics based on susceptibility to denaturation and enzymatic degradation could determine whether an allergenic condition develops or not.
In a wider context, adverse reactions to food are currently divided in toxic or non toxic. Non toxic reactions are divided in non immune-mediated, such as enzymatic-caused and drug-caused reactions, and immune-mediated, such as IgE-mediated, non IgE-mediated and mixed-immune reactions (28).
In humans, food allergy is frequently caused by IgE-mediated mechanism. Although, delayed reactions involving the digestive tract or the skin, may occur, triggered by less clear immunological mechanisms (29) possibly developed through allergen-specific T-cell activity, which may, per se, trigger an allergic inflammation process (30).
The real prevalence of food allergy is certainly higher than identified in double-blind studies. In a study made by the European Community Health Survey, in which 17280 adult human individuals, the mean of allergy or food intolerance referred was 12%, with a minimum of 4,6% in Spain and a maximum of 19,1% in Australia. The most frequently involved food varied from one country to another (17), also conditioned by cultural influences on diet (31, 32). The way of contact also appears as a conditioning factor to sensitization and allergic reaction (13).
As preliminary conclusion, more or less frequently, a

large variety of substances may act as sensitizing agents, depending on genetic factors and on allergen availability and concentration, in relation with environmental factors.

CLASSIFICATION OF ALLERGENS

Recognized and characterized allergens are subjected to a nomenclature from the Allergen Nomenclature Subcommitee of the International Union of Immunological Societies (IUIS), containing a list of more than 4000 allergens and about 200 isoallergens (33).

The name of an allergen is composed by the three first letters of it specie taxonomic affiliation Generum followed by the first letter of the specie and the Arabic numeral of the correspondent allergen (ex: Dac g 1, for allergen number one of Dactylis glomerata grass; Der p 2, for allergen number two of Dermatophagoides pteronyssinus mite).

Allergens are further reunited in groups, according to similarities between them. Following the criteria, allergens presenting close molecular weight (MW) or isoelectric point (pI) and a sequential homology ?67%, define a group in spite belonging to different taxonomic Genera. In one single specie an allergen may be composed by several molecules showing the same MW but different pI. These different molecules are designated allergen isoforms or simply isoallergens. For the example, Amb a 1.0101 represents the subtype 1 from isoallergen 1, from the allergen group 1 of Ambrosia artemisiifolia (34).

An allergen may also be classified according to it frequency of recognition in a sensitized population. It is then called a major or a minor allergen if it is recognized by, respectively, more, or less than 50% of the sensitized individuals (34). For many allergen molecules it is already known their complete sequence of cDNA and three-dimensional structure, which allowed grouping them in a small number of structural protein families, independently from their biological source. From this grouping, 28 main groups of proteins from several sources, showing cross-reactions, had result. In 6 of those groups there are allergens belonging to a few families of plant proteins related with defense against infections by fungi, bacteria and virus, or environmental stress (18). Hence, plant strands expressing higher levels of these proteins, being more resistant to environmental stress or specific diseases tend to be selected for agriculture proposals, which may contribute to an increase in cultivated plant allergenic capability (35). Another 11 groups showed sequential homology with a large variety of enzymes, like proteases, glycolitic enzymes, superoxid-dismutase, carbohydrases and esterases. Other allergen groups are composed by transfer proteins, protease-inhibitors, regulatory, structural and storage proteins (18).

POSSIBLE ALLERGENIC MECHANISMS

For some allergens it has been suggested that their enzymatic activity could function as an allergenic promoter. One example of this is mite inhalant protease Der p 1, that may increase the permeability of the respiratory mucosa, promoting a closer contact with intestine immune structures (36, 37), or act through anti-suppressive mechanisms close to the ones presented by helmintes (26, 27). Enzymatic allergens may also act as sensitization adjuvants for other non-enzymatic allergens. According to Gough et al. (1999) (27) and Wan et al. (1999) (37), Der p 1 allergenic protease, facilitating bronchial epithelium permeability, would conduce to an increase in IgE synthesis by breaking the low affinity receptor for IgE (CD23) in B-cells and monocytes. This phenomenon would possibly be associated to a decrease in T helper 1 cells (Th1) proliferation following the break of the receptor for interleukin 2 (CD25).

As hypothesis, we may say that an antigen may become an allergen by avoiding the activation of T helper 2 cells (Th2) deviation suppressor mechanisms, like the ones associated to CD8 T-cells and Th1-primed (20). Nevertheless, the extension of enzymatic activity or other biochemical functions involved in the sensitization and clinical reaction process remains to clarify (38).

CLINICAL DIAGNOSIS FOR ATOPIC DOG

Human and dog atopic dermatitis present several similarities (39). Diagnosis is based on the presence of, at least, part of the clinical criteria strongly associated to the disease, in conjunction with the elimination of several other causes of similar clinical dermatitis (40).

DIAGNOSTIC CRITERIA OF ATOPIC DERMATITIS

Willemse proposal for the clinical diagnosis of atopy (41) has been used since 1986 (table I), based on the criteria from Hanifin and Rajka (42) for the diagnosis of human atopic dermatitis. According to that proposal, 3 major and 3 minor features should be present to consider a dog as an atopic individual. Nevertheless, no assays were developed to validate those criteria.

Table I. Clinical Diagnosis of Atopy

Major features
- Pruritus
- Typical morphology and distribution:
  - (1)facial or digital involvement or
  - (2)liquenification of the flexor surface of the tarsal joint and/or extensor surface of the carpal joint
- Chronic or recidivant dermatitis
- Individual or family history of atopy
- Breed predisposition

Minor features
- Onset of symptoms before 3 years
- Facial erythema and cheilitis
- Bacterial conjunctivitis
- Superficial staphylococcal pyoderma
- Hyperhidrosis
- Immediate positive intradermal test to inhalants
- Increased serum allergen-specific IgE

As was happening in human medicine with Hanifin and Rajka criteria (42), Willemse proposal for veterinary medicine, besides the lack of validation, presented several handicaps (43) some showed lack of specificity (chronic dermatitis), others a very low frequency (hyperidrosis) and others a high frequency in non atopic dogs (pyoderma or conjunctivitis).

A few years later, in 1994, Williams et al. (44-46) proposed for human medicine a new group of criteria that were recognized in the UK as most simple and reliable. As well, in veterinary medicine Prélaud et al. (43) proposed in 1998 a new group of criteria for the diagnosis of canine atopic dermatitis. According to their study, the presence of, at least, 3 major features (table II) in a pruritic dog, after having discarded ectoparasitosis, showed a diagnostic sensitivity and specificity around 80%. This implicate that for a diagnosis of atopy a dog should present 1) pruritus and 2) should have been discarded the possibility of mange by skin scrapings or, in case of a possible sarcoptic mange, after a therapeutic assay with avermectins or other efficient antisarcoptic treatments.

Another outstanding feature is that, according to this study, this method failed to detect 20% of atopic dogs (with less than 3 criteria) and 20% of individuals falling within the 3 criteria revealed to be non-atopic. The majority of these non-atopic dogs represent probable cases of adverse reactions to food, as we will see further ahead.

Table II. Major Features in a Pruritic Dog

- Appearance of pruritus between the ages of 6 months and 3 years
- Corticosteroid-sensitive pruritus
- Bilateral cranial erythematous pododermatitis in front limbs
- Erythema of internal pinnae
- Peribucal erythema / cheilitis

Prélaud et al. (1998) (43) also included a group of minor features (table III), which presence should conduct to the suspicion of a possible atopic dermatitis. Nevertheless, besides their high specificity, some of these clinical conditions were frequently not diagnosed as linked to atopy because of their low discriminative clinical paradigm, as lesions of the elbow wrinkles, or showed an infrequent presentation, as hives. Unfortunately the criteria proposed by Prélaud et al. were also not validated by other scientific studies.

Recently, Poãta and Svoboda (2007) (47) assessed Willemse and Prélaud criteria, establishing their sensitivity in 72,3% and 68,1%, respectively, with no significant statistical differences between them. Nevertheless the reliability of their methodology and results could be questioned because only 25,5% of the individuals were subjected to elimination diets and some outpatients were included before the publication of Prélaud's diagnostic criteria.

After all, because of the high variability of the presented signs through the different patients, no clinical criteria proposal will be unfailing as diagnostic method (39).

Table III. Minor Features in a Pruritic Dog

- Predisposed breed or family background
- Chronic or recidivant dermatitis for more than 2 years
- Dull coat
- Elbow wrinkle affection
- Lick dermatitis
- Hyperhidrosis
- History of hives or angioedema
- Seasonal worsening
- Worsening on the grass
- Changing of symptoms according to the place

ATOPIC DERMATITIS INDUCING FACTORS

In humans suffering from atopic dermatitis, among inducing factors there are aeroallergens (dust mites, pollens, dander, etc) food allergens, staphylococcal superantigens and even autoallergens (48). Towards dog atopy, these allergen sources seem to play also an important role. Hence, the "Internacional Task Force on Canine Atopic Dermatitis" sustained the concept that food components could induce atopic dermatitis in some dog individuals; although this concept should not be misinterpreted, since food allergy and atopic dermatitis does not configure a single pathology (49).

Above all, it is very important for a correct clinical diagnosis in atopic dogs to evaluate the presence of other concurrent hypersensitivities and also any infectious complications

ATOPIC DERMATITIS AND ADVERSE REACTIONS TO FOOD

Upon the definition of diagnostic criteria, another outstanding feature is that atopic dermatitis and adverse reactions to food are not possible to distinguish by clinical methods, since lesion aspects appear to be quite similar (50-52). For differentiation between both dermatitis it is very important to submit the animals to a dietary restriction test (50, 51), consisting in feeding the suspected individuals for a large period of 8 to 10 weeks (53, 54), with a strict diet containing no protein that has been ingested before. Usually, a diet based on a single protein source (ex: horse, rabbit or ostrich) plus a unique source of carbohydrate (ex: rice or potato) is used (55), which is known as homemade diet.

If clinical condition improves during the dietary test it is needed to reintroduce the old diet as a provocation test (50). Following this course, if condition falls again a dietary component is proved in the dermatitis triggering. If the owner does not accept this type of diet, commercial formulations based on hydrolyzed protein sources could be used. Nevertheless, there are patients who does not respond to these commercial diets and do it to homemade ones (55, 56).

An important fact to have in mind for the diagnosis of

atopy is that there are dogs simultaneous atopic and with adverse reactions to dietary components. In these cases a clinical improvement is frequently observed during the restriction diet course without a complete clinical healing. Several recent studies have pointed to figures reaching 21 to 33% of the affected dogs (manifesting atopy or adverse reaction to food) (56-58), while others point to just 4% (59). In humans these figures reach 40% of children with moderate to intense atopic dermatitis (they have simultaneous cutaneous reactions induced by food) (50).

After all, facing a patient with a possible diagnosis of atopic dermatitis with perennial symptoms, it should be considered necessary to perform a restriction dietary assay, in order to detect if clinical manifestations, or part of them, are due to any dietary component.

ATOPIC DERMATITIS AND ALLERGIC DERMATITIS TO FLEA ALLERGENS

Like what happens with food adverse reactions, numerous atopic dogs from humid and temperate or warm climatic regions suffer simultaneously from flea allergy dermatitis. Up to one third of these dogs suffer from both types of dermatitis (47, 60).

In spite of allergy to fleas being clinically distinguishable from atopic dermatitis, in all atopic dogs a strict flea control should be performed (61), apart from cases with very inappropriate environmental conditions to flea life cycle development. For an effective control, an effort of flea eradication should be done in the animal and his cohabitants, and also in their environment. Only following these procedures a positive clinical diagnosis towards allergy to fleas will be made possible.

ATOPIC DERMATITIS AND CONCOMITANT INFECTIONS

Two of the most frequent complications of atopic dermatitis are pyoderma due to Staphylococcus sp infection and dermatitis due to Malassezia sp. An important component of pruritus is the overgrowth of microbial population in the skin. Hence, it is fundamental to evaluate their presence in skin lesions.

Overgrowth of microbial populations in canine dermatitis has been described for a few years, characterized for an elevated bacterial population, mainly S. intermedius, but also yeasts like M. pachydermatis, what is frequently seen in allergic patients (62). Frequent and recurrent staphylococcal pyodermas has been described for a long time in atopic dogs (63) and a higher adherence from these agents to atopic dog keratinocytes when compared to non atopic ones was proved (64). A close condition was also found in humans, where 90% of atopic lesions show a great increase in the number of S. aureus (65), and in which a relation between staphylococcal enterotoxins and the severity of skin lesions was found. These enterotoxins were proved to act as allergens to humans (66) and also as superantigens (65), inducing a powerful and non specific activation of lymphocytes which cause cytokine production and the amplification of the cutaneous inflammatory response (63). For dogs, it has been estimated that 25% of S. intermedius secrete superantigen-capable exotoxins (67). Another important fact is that 39% of atopic dogs without visible pyoderma showed positive response to antibiotherapy (68).

With regard to M. pachydermatis, several studies point to a probable liberation of antigens that may penetrate the skin, especially in atopic dogs (69). These and several other reports show the probable existence of an IgE-mediated reaction to Malassezia sp in dogs (63).

To evaluate bacterial and yeast populations in skin lesions it is used the cytological method, since it is simple and quick (70) and the scotch tape method is the one that most microbial populations identifies (62, 71). For counting it is recommended to use the immersion oil objective (x1000) observing at least 20 microscope fields (62). The presence of more than 5 bacteria or 2 yeasts per field suggests a microbial overgrowth (62).

As for summary, the clinical diagnosis of an atopic patient requires:

1.To discard ectoparasitic dermatitis, firstly.

2.The presence of, at least, one part of the clinical criteria strongly associated to atopy.

3.To determine if other components (food or fleas) of allergy are present.

4.Evaluation of possible microbial complications.

LABORATORY DIAGNOSIS – IMPORTANT FEATURES FOR INTERPRETATION

Actual position regarding canine allergy should be based on the recent and of considerable interest nomenclature proposed by the "American College of Veterinary Dermatology Task Force on Canine Atopic Dermatitis" that points to a difference between (72):

•Canine atopic dermatitis: A genetically predisposed in?amatory and pruritic allergic skin disease with characteristic clinical features associated with IgE antibodies most commonly directed against environmental allergens.

•Canine atopic-like dermatitis: An in?amatory and pruritic skin disease with clinical features identical to those seen in canine atopic dermatitis in which an IgE response to environmental or other allergens cannot be documented.

These focus stands as the basis to be pursuit for the diagnostic course from clinics to laboratory, as follows.

As we have already seen, for allergy diagnosis in vivo and in vitro methods are currently applied. General accepted semiotic course for this proposal is: 1 – Detailed and directed anamnesis; 2 – Clinical examination; 3 – Intradermic skin tests; 4 – Total and specific IgE determinations; 5 – Restriction and provocation tests. Although, other complementary methods are referenced as very useful for diagnosis clarification, especially in what concerns to food allergy, like Western Blotting, evaluation of basophile activation (Basophile Activation Test – BAT and Flow Cytometric Basophil Allergen Stimulation Test – FAST), liberation of cysteinyl

leukotrien C4 (LTC4), plasmatic histamine and eosinophil cationic protein (ECP) determination, fecal IgE determination, specific IgG4 and RAST inhibition (73).

Cutaneous tests are the classical example of an in vivo method for the search of specific IgE to different allergenic sources, remaining of outstanding importance because of their high sensitivity for the detection of IgE-mediated hypersensitivity reactions (74). In human medicine the most used variant is the skin pick tests (SPT), while in veterinary medicine the most accurate is the intradermal technique (ID). SPT for food allergens present a higher sensitivity (>90%) than in vitro search of specific IgE, but specificity stays around 50%. The negative predicted power is high (95%), but positive results show a lack of clinical relevance. Concerning the diameter of the obtained weal flare, it is considered as predictive of the occurrence of a clinical reaction following ingestion, although with no value to predict the severity of the reaction, neither to estimate the minimum triggering doses (17).

Double-blind oral provocation tests are considered for humans as the gold standard for food allergy diagnosis in individuals older than 3 years old, with a probability of false-negative occurrence of just 1-3% (17). With animals the "double-blind" is obviously unnecessary but, like for humans, it must be very strictly designed to allow the validation of results. Owners should be well convinced of the importance of the test to strictly persist with the restriction diets for enough time. One thing we must have in mind is that in presence of a previous history of severe clinical reaction with a suspected food, provocation test should be clearly avoided (17). Even skin tests are not completely free of risk, especially in highly sensitized patients (75). Hence, in spite of being an easy technical procedure, it should be done in a proper clinical environment.

Within in vivo diagnostic methods, epicutaneous tests, showing a high specificity in spite of a low sensitivity, may also be very useful to identify delayed hypersensitivity reactions (type IV). These tests should also be performed with good criteria because skin is also a way of sensitization. A natural example of that is the development of type I hypersensitivity to fleas in dogs, in the course of repeated stimulation where the observed reaction was initially of type IV (76). In fact, for the diagnosis of delayed hypersensitivity reactions with origin in haptens, such as nickel, cobalt or chromium salts, patch-test may also be very useful for diagnosis (77, 78). This could be very helpful for diagnosis of contact allergic reactions to objects, such food recipients or collars, including buckles.

In vitro methods offer the advantage of precise quantification, safety, less possibility of drug interference and a better technical profit, because many sera can be tested at the same time and may be kept frozen for further future determinations. The use of modern available in vitro techniques is also providing a better cost/sensitivity compromise with an appreciate progress in specificity, as monoclonal antibodies and recombinant allergens are entering the scene.

For IgE determinations, several methods may be used, from ELISA or ELISA-derived methods to other more sophisticated and commercially available. First generation home-made methods require special calibration in order to produce accurate results. Calibration should begin at the proper allergenic extracts according to the International Standards (IS), based on reference extracts kept in the National Institute of Biological Science and Control (NIBSC), in the United Kingdom (34). That is, in fact, very important because determined IgE levels depend on anti-IgE antibodies used for the detection and on the allergens fixed to the plate wells by coating.

According to Hamilton & Adkinson (2004) (74) there were three generation of standardized commercial methods for laboratory diagnosis, allowing total and specific IgE determination. In the first generation there were several methods such as: Multiple Antigen Simultaneous Test (MAST CLA® – Hitachi Chemical Diagnostics, EUA); Hycor Hy-Tech EIA and Thabest IgE, achieving positive/negative or semi-quantitative (in classes) results. In the second generation there were methods like CAP System® (Pharmacia Diagnostics) and AlaSTAT® (Diagnostic Products Corporation – DPC, EUA), which were already semi-automatic and presented a high accuracy of quantization with a good analytical performance. Third generation includes methods like UniCAP® (Phadia) and Immulite® (DPC), highly automated and providing high precision determinations with a very well defined positive baseline. Although, concerning food allergy, the agreement for specific-IgE positive results between CAP System® and AlaSTAT® reached only 80% (79).

Other tests for specific-IgE screening are very useful for the diagnosis of atopy, detecting the presence of specific-IgE for multiple allergenic sources, which in case of clearly positivity will support the hypothesis of atopy. These tests include a panel of up to 15 allergenic specificities representing the most common allergen sources for adults or for children, with variants for aeroallergens and food allergens, according to conditions as, for instance, the pollinic prevalence or the ingestion habits of different societies. The most spread screening test is possibly Phadiatop® (Phadia) that in case of a negative result will reveal the absence of atopy in the suspected individuals (74). In human pediatrics it serves for an early identification of an ability to develop an allergic disease. Although, it should be present that the prevalence of specific-IgE always stands above clinical allergy. For qualitative identification of recognized allergens there is also available an important range of commercial blots from different allergen sources – AlaBLOT® (Diagnostic Products Corporation – DPC). Although, for each source, the huge amount of different potentially allergenic proteins, which presentation may substantially vary with aspects such as specie and variety, efficiency of allergen extraction and their standardization, separation principles (ex: IEF, SDS PAGE, or double-dimensional) and transference to immune-fixation membranes. Hence, it is sometimes necessary to perform a

laboratory home-made separation to obtain the blotting membranes for research proposals. In fact it is frequently necessary to confirm if there is a cross-reaction between two allergen sources, by RAST (radioallergosorbent test) inhibition assays and what allergens are implicated in the cross-reaction, by Western Blotting inhibition.

The total set of recognized allergens from a given source is denominated alergome, while spectrotype defines the set of allergens recognized by each patient. Patient spectrotypes are obtained from IgE recognition of separated allergens over fixation membranes. Separation of allergenic proteins may be performed through different biochemical concepts like pI, by isoelectric focusing (IEF), or molecular weight, by SDS PAGE (12, 80, 81). The sum of all spectrotypes will, then, expose the allergome. The adequate process for those identification and characterization is the Western Blotting on immune-fixation membranes obtained from double-dimensional separations (12, 82).

Other methods based on cellular response are also available for specific IgE detection. One of them is based on the release of inflammatory mediators by basophile, such as histamine and LTC4 in the presence of the suspected allergens involved or anti-IgE antibodies that will fix to surface IgE molecules. These methods may stand as a complement to immunochemistry serum determinations, presenting although a further time-consumer and delicate execution. The variability in basophile mediators release, following allergen stimulation through the different patients, also limits it diagnostic value (83, 84). On the other way, in the presence of sensitization and according to the stimulating agent, histamine-releasing test shows different levels of response (85, 86, 87) and specificity levels also present a considerable variation when compared to skin tests (86, 88). Another method based on cellular study is CAST® (Cellular Antigen Stimulation Test) – ELISA (Buhlmann Laboratories). It measures the liberation of LTC4 from allergen-stimulated basophiles, with sensitivity in humans, of 18% for acetylsalicylic acid (86) and 85% for several food allergens (89). Regarding specificity, it reached 67 and 100%, respectively. The advantage for diagnosis relies on the identification of adverse inflammatory reactions to certain drugs and food additives by non IgE-mediated mechanisms (pseudo-allergies), although the reduced sensitivity for certain substances brings important limitations to the method (74).

The evaluation of IgE-mediated cellular response may also be accessed by studying CD63 expression of IL-3-primed activated basophiles, detected in flow cytometry, following whole-blood culture in the presence of several possible allergens. In this case, the observed percentage of activated basophiles has to be corrected concerning the spontaneous basal CD63 expression. Another feature that should also be accessed for a reliable interpretation of this method, avoiding false-positive results, is platelet (also CD63-expressing elements) adherence to activated basophiles (74).

In spite of the referred tests, either strictly biochemical or also related to basophilic response, all observations only consists in a small part of the possible prospective information, since there are frequent cases of specific-IgE for several allergen sources without clinical allergy, and those levels are not a predictive factor for allergy.

Additional contribution to understand several allergy-triggering mechanisms may be obtained by characterizing different lymphocyte subpopulations pattern of cytokine response under stimulation, either in vivo (following provocation tests) or in vitro. For cytokine detection, several methods have been tested, from ELISA and flow cytometry with anti-cytokine specific monoclonal antibodies, to RT-PCR (reverse transcription-polymerase chain reaction) for cytokine mRNA, with many variants of these methods being used to test the response to several substances, either allergenic or not.

The huge diversity of standardized methods available for diagnosis in humans, with advantages and handicaps between them, require accuracy and sometimes costly procedures to be used for other species diagnosis, and finally limits the use for this proposal. Nevertheless, worldwide spread methods like UniCAP® or even AlaSTAT®, would be very useful for veterinary allergy diagnosis after the necessary gauging procedure.

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