

Original article

Complementary roles for lipid and protein allergens in triggering innate and adaptive immune systems

Background: Recent advances in allergy research mostly focussed on two major headings: improving protein allergen purification, which is aimed towards a better characterization of IgE- and T-cell reactive epitopes, and the potential new role for unconventional innate and regulatory T cells in controlling airway inflammation. These advancements could appear to be in conflict each other, as innate T cells have a poorly-defined antigen specificity that is often directed toward nonprotein substances, such as lipids.

Method: To reconcile these contrasting findings, the model of cypress pollinosis as paradigmatic for studying allergic diseases in adults is suggested.

Results: The biochemical characterization of major native protein allergens from undenatured pollen grain demonstrated that the most relevant substance with IgE-binding activity is a glycohydrolase enzyme, which easily denaturizes in stored grains. Moreover, lipids from the pollen membrane are implicated in early pollen grain capture and recognition by CD1⁺ dendritic cells (DC) and CD1-restricted T lymphocytes. These T cells display Th0/Th2 functional activity and are also able to produce regulatory cytokines, such as IL-10 and TGF- β . CD1⁺ immature DCs expand in the respiratory mucosa of allergic subjects and are able to process both proteins and lipids.

Conclusion: A final scenario may suggest that expansion and functional activation of CD1⁺ DCs is a key step for mounting a Th0/Th2-deviated immune response, and that such innate response does not confer long-lasting protective immunity.

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Adult onset of allergic diseases has increased during the last quarter of the 20th century and recent birth cohorts are more likely to become atopic than individuals born in the mid-20th century (1). The contemporary fall in the incidence of paediatric infectious diseases on account of obligatory vaccinations and widespread use of antibiotics (2), and cleaner lifestyles are considered factors underlying an allergic response to ubiquitous inhaled antigens by the immune system of genetically susceptible individuals (3–5). Several questions still remain open in the field of interactions between the innate and adaptive immune systems in response to exposure to allergens; particularly those concerning pollen immunobiology and relation with the environment, nature and function of allergen-reactive T cells, timing of the allergic response, and how we can interfere with such processes.

One of the initial steps in allergy development is generation of allergen-specific CD4⁺ helper T cells. Upon encounter with inhaled allergens, naïve T cells under the influence of interleukin-4 (IL-4) differentiate into Th2 cells (6). Allergic individuals have a high proportion of Th2 IL-4-producing clones and a low proportion of Th1 interferon- γ (IFN- γ)-producing clones, while nonatopic

individuals have allergen-specific T-cell clones that were preferentially of a Th0/Th1 phenotype (7). In some studies, bronchoalveolar lavage (BAL) fluid T cells from atopic asthmatics were predominantly Th2, unlike those from nonatopic asthmatics (8). Experiments in murine models and other clinical studies did not always confirm these findings (9, 10). Even though the inherited propensity of allergic individuals to mount a Th2 response upon encounter with an allergen plays a critical role in the pathogenesis of allergy (11, 12), response regulation appears more complex than merely a postnatal switch of the two functional T-helper cell subsets (13, 14).

Selective allergen recognition depends on B- and T-cell receptor (TCR) molecules that bind specific regions of the antigen molecule, termed epitopes. More than 4000 substances, the vast majority of which are proteins, mostly enzymes, have been identified to date as allergens—that is common substances in the environment that elicit an IgE-mediated immune response in a genetically predisposed individual (see lists at <http://www.allergen.org> and/or <http://www.allergome.org>). Despite research into peptide allergen characterization designed to improve the quality of allergy extracts, poor clinical results

were obtained from commercialized products for immunotherapy (15–17). Indeed, the source material is not always guaranteed as 'freshly collected' and sufficient amounts of proteins often cannot be extracted from industrially collected pollen (18).

In this review, we analyse experimental findings from studies on the cypress allergy model, in an attempt to understand in depth immune dysregulation in allergic diseases.

The cypress allergy model

Although cypress pollen counts during the winter (January–March) are usually two- to fivefold higher than *Graminaceae* pollen counts in the spring, and are found in the atmosphere for 3 months as opposed to 2–3 weeks, clinically evident cypress allergy (conjunctivitis and rhinitis) is confined to a small adult population and is rare in children (19). Large studies on unselected young adults in France (20) and Italy (21) estimated the prevalence of cypress allergy around 2.4–8% of the general population (22). Most adults with recent-onset cypress allergy do not have a familial predisposition for atopy and are often sensitive only to cypress pollen; in addition, sensitization seems to need long-lasting exposure (23). Unlike classic seasonal allergy, a more complex mechanism, perhaps related to the nature of pollen itself, appears to underlie cypress allergy. It might involve nonprotein pollen constituents, some unconventional T cells and antigen-presenting cells (APCs).

Pollen grain capture

Mechanisms underlying immune recognition of pollen grains as allergens have long been under debate, mainly because of the biochemical diversity of allergens and their localization within the pollen grain (24). As most protein-sensitizing agents reside in the exine membrane and/or in the cytoplasm (25), the rapidity of delivery to the immune system *in vivo* remains inexplicable. Traditional models suggested that nonspecific receptors such as C-type lectins and integrins rapidly bind surface glycoproteins and favour alveolar macrophage phagocytosis of the whole pollen grain and/or pollen starch granules (<5 µm) (26). T-cell receptors may interact directly with antigen-derived peptides that are associated with major histocompatibility (MHC)-encoded antigen-presenting molecules (27).

Peptides are not the only target of T-cell response, as T cells recognize a variety of lipid antigens by means of a family of nonpolymorphic genes (CD1) (28) that evolved to present lipids and glycolipids to the mammalian immune system (29, 30). The respiratory system contains macrophages and dendritic cells (DCs) (31, 32), which play a central role in T-cell priming and effector

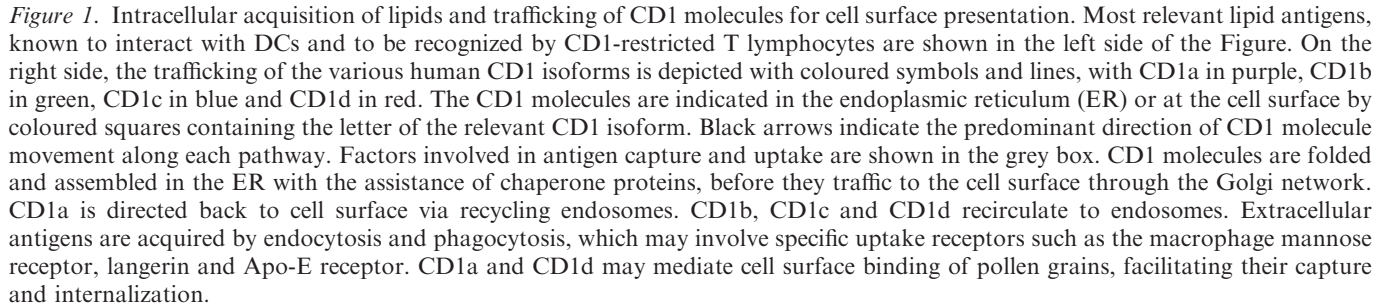
responses to inhaled allergens (33). Both cell types have the potential to express CD1 proteins. Interestingly, CD1a is frequently over-expressed in pulmonary DCs and in BAL suspensions from allergic subjects (34).

We investigated whether CD1 protein expression was implicated in pollen capture and processing *in vivo* and *in vitro* (35). Membrane phospholipids (PLs) mediate rapid contact between inhaled cypress grain and mucosal DCs. In electron microscopy studies, pollen membrane PLs [particularly phosphatidylserine, cardiolipin, phosphatidyl-ethanolamine (PE) and phosphatidyl-choline (PC)], appeared as nonbilayer hexagonally packed cylindrical protuberances on the membrane surface (36). The exposed PL acyl side chains entered the CD1 pocket, thus stabilising pollen grain-DCs contact. The CD1⁺ DCs/pollen grain interaction was blocked by preincubating DCs with antibodies directed against the cell surface-expressed CD1d and, to a lesser extent, CD1a proteins. Nonbinding control Ig or monoclonal antibodies, specific for MHC class I, CD1b or CD1c molecules had no effect. Confocal microscopy revealed that the whole pollen was rapidly ingested within a few minutes, and outer membranes were progressively concentrated and digested within DCs cytoplasmic vesicles (37).

This process accounts for *in situ* expansion and activation of mucosal CD1⁺ APCs in allergic inflammation (35, 37, 38) (Fig. 1), and provides evidence in support of their primary role in recognizing nonprotein constituents of allergens (39, 40). Indeed, pollen-associated lipid mediators, such as phytosteranes, modulated some DCs functions *in vitro* by inhibiting IL-12 p70 production and potentiating their Th2 polarizing capacity toward naïve T cells (41). They also recruited and activated different cell types, such as polymorphonuclear granulocytes, before the T-cell-mediated adaptive immune response was triggered (42, 43).

CD1-restricted PL-specific T lymphocytes, which include CD4⁺ or CD8⁺ TCRαβ⁺ T cells, CD4⁺CD8⁺ TCRαβ⁺ T cells, Vα24⁺ iNK-T cells, as well as TCRγδ⁺ T cells, responded promptly to lipid-antigen recognition by secreting a wide array of cytokines, including high amounts of IL-4, and expanded at mucosal allergic inflammation sites (12, 37, 44, 45). Responses to pollen PLs were maximally evident in blood samples from allergic subjects during the pollen season, were occasionally seen in nonallergic subjects, and were always absent in *Mycobacterium tuberculosis*-exposed health-care workers (37). In addition, nasal mucosa-derived γδ T-cell clones from allergic subjects also recognized pollen-derived PE in a CD1d-restricted fashion (46).

It may be speculated that the early allergic response initiates with primary mucosal recognition of pollen grain lipids by CD1⁺ DCs and CD1-restricted T cells, which ensure rapid handling of the foreign inhaled grain. As CD1 molecules also present protein substances to T cells, two different kinds of antigen recognition may coexist. Indeed, intact pollen grain is degraded in the lysosomal



compartment where both CD1 and MHC class II molecules are present (47).

Innate immune system allergen recognition in the respiratory tract might be the same in allergic and nonallergic subjects, because different cell types interact directly with pollen grain, independently of IgE (48, 49). The recognition pattern frequently differs from one patient to another, and many respond to multiple epitopes within a given allergen. Immune mapping studies showed the targets of natural immune responses have a broader distribution than was previously appreciated, with more

T-cell epitopes from antigenic peptides are presented to CD4⁺ TCRαβ⁺ cells by APCs bearing various types of MHC class II-DR (51), -DQ (52) and -DP molecules (53). Dominant allergenic epitopes have been characterized for more than 360 peptides derived from 54 allergens (see Allergome, etc.). In the *Cupressaceae* family, only Jun a1 from *Juniperus ashei*, Cha o1 from Japanese cypress, and Cry j1 from *Cryptomeria japonica* were cloned and tested for immunodominant T-cell epitopes in sensitive subjects (54, 55). Several dominant and sub-dominant epitopes were identified, most of which were also cross-reactive, or surprisingly reactive in clinically unsensitized subjects (55).

As effector (allergen-specific Th2) and suppressor (allergen-specific Tr1) T cells co-exist in healthy and allergic individuals, their ratio determines the development of healthy or allergic immune response, as well as the outcome of specific immunotherapy (56). In healthy individuals, IL-10-secreting allergen-specific T cells (Tr1) predominate; in allergic patients, there is a shift towards IL-4-secreting T cells-Th2-type cytokine production.

In the past few years, knowledge of T-cell subpopulations has expanded the model beyond the Th1/Th2 paradigm and many investigators have re-assessed 'suppressor T cells', which negatively regulate the immune system. The first described subset was the so-called naturally occurring regulatory T cells (Treg) (57). Regulatory T cells originate in the thymus and appear to be critical in maintaining tolerance to auto-antigens. Thymectomy of mice a few days after birth resulted in failure of Tregs to appear in the periphery, and animals developed a multi-organ autoimmune disease. CD4⁺ CD25⁺ is the hallmark phenotype of naturally occurring Tregs which also express the forkhead (winged helix) transcription factor Foxp3 (58). Naturally-occurring Tregs may regulate allergic diseases (59), and interestingly, an X-linked recessive syndrome characterized by autoimmunity and cutaneous allergy has been linked to mutations in the Foxp3 gene (60). As Tregs can be isolated from unmanipulated animals and unimmunized individuals, their TCR specificity does not seem to be directed against foreign allergens, but rather against a variety of self antigens (61). Even though the frequency of single allergen-specific Tr1 cells, which are also CD4⁺ CD25⁺, ranges from 1 in 1000 to 1 in 20 000 of the whole CD4⁺ CD25⁺ Treg population, Tr1-Tregs suppress Th1 and Th2 responses, as well as responses to allergens (62).

Inducible Treg cells are not present in naïve individuals, but appear only after antigen exposure and have immunogenic specificity. Inducible Tregs act predominantly by secreting regulatory cytokines such as IL-10 and/or tumour growth factor- β (TGF- β ; Th3 cells) and do not require cell-cell contact to mediate suppression (63, 64). Given these properties, they seem more likely to be candidate regulators of the immune response than naturally occurring Tregs (65).

A great deal of uncertainty remains with regards to differentiation factors, antigen specificity and mechanisms of action of inducible Tregs, which seem to vary with the experimental model. Surprisingly, during protozoan infection and chronic cutaneous leishmaniasis, Th1 cells emerged as the main producers of IL-10, the anti-inflammatory cytokine (66, 67). In dermal lesions from mice infected with *Leishmania major*, IL-10 was produced by innate immune cells and also by CD4⁺ CD25⁺ Foxp3⁺ Th1 cells. Production of IL-10 by antigen-specific Th1 cells may represent a feedback control mechanism to limit damage to the host caused by a strong Th1-polarizing condition, while maintaining an effector response. In addition, exposure of mature pulmonary DCs to respira-

tory allergens stimulated the development of Tr1-like cells, which are dependent on DC IL-10 production (68, 69).

T helper type 1 cytokines that are produced in response to repeated stimulation of toll-like receptor (TLR) and/or other receptors on cells of the innate immune system exert a marked suppressive effect on allergen-specific responses. Bacterial nonprotein substances (often containing lipids) suppress airway eosinophilia, reduce IL-4 and IL-13 production, or redirect Th1/Th2 imbalances via IL-12 or IFN- γ production (70). In addition to naturally occurring and inducible Tregs, which suppress reactivity against self antigens, heterogeneous populations of innate and adaptive allergen-specific Tregs might be conditioned by environmental stimuli to act preferentially at the mucosal sites during allergen encounter.

Phospholipid-specific T-cell clones derived from cypress-sensitive subjects exerted regulatory activity. While examining lipid antigen reactivity of T-cell clones, antigen specificity was found against PC 16 : 0/16 : 0, a species, which is not part of the PL mixtures extracted from cypress pollen. One CD4⁺ TCRV α 24⁺ clone produced discrete levels of IL-10 and TGF- β 1 in response to stimulation by synthetic disaturated (16 : 0/16 : 0) PC, brain and egg PC mixtures. When pulsed with stimulating PLs, they acquired a mainly IL-10 and TGF- β 1 cytokine secretion pattern (71).

The initial reactivity against pollen PLs may recruit T cells with higher avidity for different PL antigens. After encountering an environmental antigen at mucosal surfaces, these T cells might physiologically protect the host from potentially dangerous dietary or inhaled products, and restrain any tendency toward autoreactivity or damaging hypersensitivity. This antigen specificity might be an example of epitope-spreading during the immune response (72). Alternatively, as PC 16 : 0/16 : 0 is the most abundant PL in the pulmonary surfactant (73), it may become an autoantigen in allergic patients. Indeed, the PL composition of human respiratory surfactant is frequently altered during allergic respiratory diseases (74, 75). Cross-reactivity between similar surfactant PL compounds containing palmitate acyl chains and PL-triggered mucosal T cells may explain worsening of airway inflammation in patients treated with natural porcine surfactant, and then challenged *in vivo* with an allergen (76).

Supporting these hypotheses are data from normal intestinal intraepithelial lymphocytes (IELs). They include PL-specific TCR $\gamma\delta$ ⁺ T-cell clones which, when exposed to synthetic 16 : 0/16 : 0 PC, 18 : 2/18 : 2 PE or 16 : 0/18:2 PG, strongly secreted TGF- β 1 in culture supernatants (77). In IELs, other autoreactive V α 24⁺ TCR $\alpha\beta$ ⁺ T-cell clones displayed basal IL-10 and TGF- β 1 secretion that was not influenced by exogenous PL stimulation. Although limited to a very small fraction of clones, it may be an innate regulatory response to lipid antigens derived from gut commensal bacterial flora and/or from lipids in ingested food. Unconventional IELs,

such as i NKT or $\gamma\delta$ T cells, may thus constitute a specialized T-cell subset which continuously checks mucosal reactivity. As they develop in a genetically programmed fashion and act by nonselectable pattern-recognition receptors, their activation does not confer long-lasting protective immunity (78).

The IgE-specific responses

Although the Th2-type adaptive response to allergens is followed by specific IgE production, airway eosinophil infiltration and bronchial hyperreactivity may occur in the absence of IgE (79). Indeed, IgE are produced in mice lacking IL-4, IL-13 and IL-4R α common chain receptor (80, 81). In T-cell deficient and germ-free wild-type mice, IgE are spontaneously produced independently of MHC class II cognate help, are not inhibited by Tregs, and increase with age even in naïve animals (82).

Most cypress-sensitive subjects do not display high levels of specific IgE toward commercially available reagents (83). As the pollen does not contain starch grains or orbicules as allergen vectors, surface-disposable allergens could be scarce (84). Polyacrylamide gel electrophoresis has been widely used to characterize proteins extracted from cypress pollen. However, as this technique is applied to a fragment of the original protein structure, its complete tertiary folding remains unknown. Moreover, as most allergens are quite similar to plant enzymes, their activity is temperature-dependent, and improper storage of collected pollen grain may cause degradation.

Pollen allergen structure is commonly studied by cloning cDNA of the fragmented peptide and by searching gene banks for homologies with known proteins (85). This technique provides no information about allergen structure and position on the pollen membrane, which are both crucial for T-cell recognition of the peptide as an allergen. Detailed biochemical characterization of native pollen extracts is needed to obtain a full proteomic map of the pollen grain envelope, in order to identify either IgE- or T-cell-reactive dominant epitopes. This strategy would constitute a major step forward in standardizing extracts for diagnosis and therapy of allergic diseases.

Alternatively, principal protein allergens could be enzymes, located at the surface of pollen grain but rapidly inactivated by starvation procedures in industrial manufacturing. Indeed, native enzymatic proteins were characterized from frozen pollen grains samples, which were collected directly from trees, thus preventing loss of allergenic potential. Staining techniques revealed the active enzyme dwelt on the pollen grain surface or inside the exine membrane, i.e. outside the cytoplasmic membrane. Native protein extracts were tested for their ability to stimulate T cells from allergic subjects and to bind circulating specific IgE. The main enzyme recognized by IgE, β -galactosidase (β -GAL), was immunoprecipitated by sera from allergic subjects (86). *In vitro* purified

β -GAL stimulated proliferation of T cells from allergic subjects and favoured emergence of CD4⁺ T-cell clones with specific reactivity to β -GAL (86).

Cross-reacting peptides do not necessarily induce clinical symptoms during naturally occurring seasonal exposure (87). In fact, a common 46 kD fraction extracted from cypress and olive pollens is co-immunoprecipitated by sera of sensitive subjects (86). However, in cypress-monosensitive patients, the cypress-derived β -GAL co-immunoprecipitated an additional 50 kD peptide. Thus, tertiary folding of the same enzyme in different plant species may account for the partial IgE and T-cell cross-reactivity in subjects who are allergic to cypress but do not manifest symptoms during olive pollination.

Lipid membrane components, particularly PLs, are a new source of antigenic substances that are recognized by T cells from cypress-sensitive subjects. Phosphatidylethanolamine (18 : 2/18 : 2) was detected by circulating IgE from a subset of allergic patients. This finding was also evident for some olive- and *Graminaceae*-sensitive subjects (46).

All these data suggest that the adaptive IgE-specific immune response toward pollen constituents involves diverse biochemical substances derived from the grain membrane. However, the wide range of cross-reactivity with peptides or PLs derived from other plants, pollens or fruits suggest mucosal T cells predominate over IgE in mediating clinical hypersensitivity.

Timing of the overall allergic response

Like the humoral reaction to T-dependent antigens, most specific immune responses appear to develop from the synergistic action of innate T cells and DCs (88). The innate immune response at mucosal sites is the first line of defence against offending agents, and local immature DCs process both lipid and protein antigens. Despite the capacity to express both MHC and CD1 molecules, only CD1 recycle between late endosomes and the plasma membrane of immature and mature DCs, which sample and present lipid antigens throughout their maturational life span (89). In this setting, DCs also direct the T-cell response toward a Th2-type cytokine secretion pattern (Fig. 2). In fact, in experimental animal models, the absence of CD11c⁺ DCs abrogated airway hyperreactivity (AHR) but not the eosinophilic inflammation after allergen challenge (33).

It is worth noting that in conventional T-cell deficient mice, eosinophilic inflammation was induced by small numbers of co-transferred $\gamma\delta$ T cells and i NKT cells, whereas either cell type alone was ineffective (90). Despite the report of an extraordinarily high frequency of i NKT cells in the airways of humans with allergic asthma (91, 92), there is still a debate over the frequency of i NKT cells in the BAL fluid of patients with mild-to-moderate asthma (93). We observed the frequency of CD4⁺

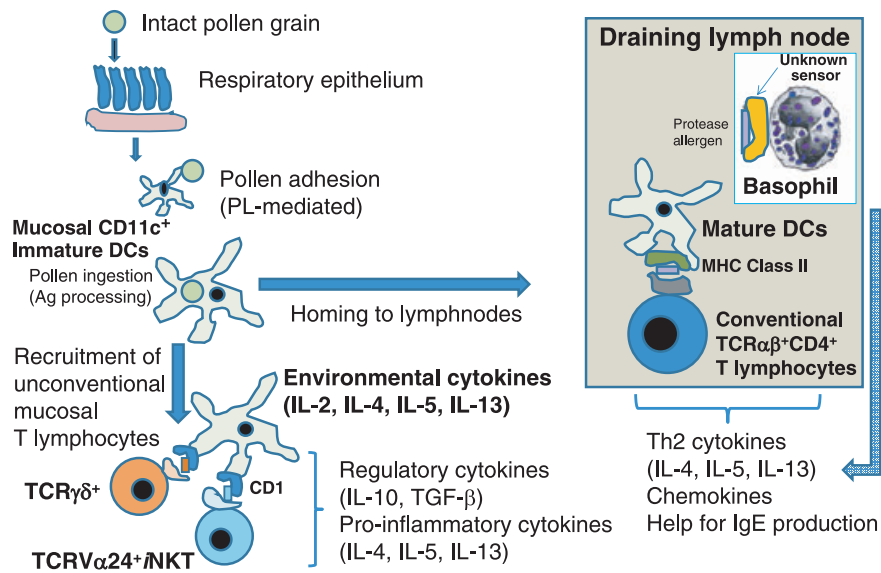
Innate mucosal allergic response**Adaptive systemic allergic response**

Figure 2. Complementary roles of innate and adaptive immune responses to inhaled pollen. Cypress-sensitive patients became symptomatic in few minutes after pollen exposure. As most of the sensitizing proteins are contained inside the intine membrane or in the cytoplasmic region of the pollen grain, it is difficult to imagine their prompt delivery to effector T lymphocytes in draining lymph nodes of the upper and/or lower respiratory tract mucosa. A multi-step pathway that reconciles most of the different effector mechanisms of the allergic reaction is proposed. In the respiratory mucosa CD1 receptors on cells of the innate immune system (principally CD11c⁺ DCs, TCR Vα24⁺ iNKT and TCR γδ⁺ T cells) rapidly capture and recognize the pollen grain membrane PLs. Environmental cytokines (mainly IL-2 and IL-5) contribute to the expansion of pro-inflammatory CD1-restricted lipid-specific iNKT and γδ T cells which, in turn, secrete large amounts of Th2-type cytokines (IL-4 and IL-13). After this initial phase, DCs and basophils migrate into draining lymph nodes, where protein allergens are presented to MHC-restricted conventional TCR αβ⁺CD4⁺ lymphocytes which amplify the allergic reaction by secreting Th2-type cytokines, chemokines, and provide the help signal for B-cell-mediated systemic IgE production.

Vα24⁺ iNKT cells usually did not exceed 5–10% of total CD4⁺ lymphocytes in the BAL fluid of asthmatic subjects (Spinozzi F, de Benedictis FM, unpubl. obs.). In other words, while it remains extremely plausible that CD1d-restricted Vα24⁺ iNKT cells play a significant role in allergic airway inflammation, other lipid-specific T cells at mucosal surfaces may have an equal or even greater role. They include CD4⁺ TCRαβ⁺ T cells and TCRγδ⁺ T cells that are either CD4⁺ or double-negative (i.e. lacking both CD4 and CD8 expression). They have the capacity to drive a lipid antigen-specific inflammatory response in cypress pollen-sensitive subjects (94).

Toll-like receptors on the surface of DCs, mast cells and other cells of the innate immune system probably play a role in the Th2-based inflammatory response, as recently showed in TLR-2 and TLR-4 knockout mouse models (95). Ligands for these receptors include Gram-negative bacteria-derived LPS, Gram-positive bacteria-derived lipoteichoic acid, other bacterial lipoproteins and glycolipids. Thus, tissue APC receptors, such as TLRs and CD1, may act in concert to amplify a local inflammatory reaction and a systemic switch to IgE production.

At later stages of the adaptive (IgE-driven) immune response, protein antigen presentation by MHC class II

may require DCs maturation (89). In fact, secondary migration of DCs to draining lymph nodes makes protein peptides fully disposable for MHC class II-restricted recognition which, in turn, initiates the adaptive immune response (Fig. 1). The adaptive response generally depends on innate T-cell-driven secretion of environmental cytokines, which follows immune recognition of the allergen. However, after subcutaneous immunization, some proteases (papain) derived from known allergens, such as Der p I, interact directly with basophils in draining lymph nodes before induction of the adaptive immune response. This recent observation emphasizes the role of the basophil as an innate initiator of Th2 responses to protease allergens (96).

Targeting innate recognition system to shut-down allergic response

New therapeutic concepts based on patient-tailored immunotherapy using recombinant allergens (97) and sublingual immunotherapies (98) are slowly moving from the bench to the bedside. Fine peptide-mapping techniques now permit experimental immunotherapy with

T-cell-based peptides. In small human clinical studies on bee venom (99) and cat allergy (100), induction of specific T-cell anergy was associated with a decrease in the allergen-specific IgE : IgG₄ ratio and increases in IL-10 and/or TGF- β (97).

Synthetic DNA sequences containing CpG motifs were covalently linked to Amb a 1-the major ragweed allergen-to generate allergen immunostimulatory conjugates. In a placebo-controlled clinical study, treatment with these agents redirected ragweed-specific Th2 responses toward Th1 responses, by increasing INF- γ , CXCL-9 and CXCL-10 expression and decreasing IL-5, CCL-17 and CCL-22 expressions (101). In the phase 2 trial, a 6-week regimen appeared to offer long-term clinical relief of ragweed allergic rhinitis (102), but no substantial differences emerged in T-cell cytokine secretion patterns in the treatment group and controls. However, effective suppression of allergen-responsive T cells in target organs (such as the lung) requires interventions aimed towards enhancing locally produced IL-10 or TGF- β (103). Studies on allergen immunotherapy showed a reduced local nasal tissue infiltration by eosinophils, basophils and Th2 T cells, and an increased number of IL-10⁺ and TGF- β ⁺ cells (104). These cytokines were not detectable outside the pollen season, thus suggesting that local mucosal contact with allergen was necessary for these effects. In the absence of allergic sensitization, IL-10 and TGF- β responses were not a feature of the normal mucosal response to pollen. As only 15–20% of these nasal IL-10- and TGF- β -producing cells were T cells, and IL-10 and TGF- β also co-localized to macrophages and DCs (40%), it is possible that local increases in IL-10 and TGF- β belong mainly from cells of the innate immune system. Therefore, a right way to interact with these cells is only partially dependent on a peptide antigen stimulation.

Indeed, as in the ovalbumin (OVA) hypersensitivity model, systemic or intranasal administration of a synthetic glycolipid of the α -galactosyl-ceramide family (KRN7000) inhibited AHR, when given to mice before challenge (105). The underlying mechanism is not yet clearly understood, given that KRN7000 is a strong activator of *i*NKT cells, which make copious amounts of cytokines, such as IL-4, IL-5 and IL-13, that are associated with worsening or induction of AHR. KRN7000 may induce a dominant Th1-type response

that negates the effects of Th2-produced cytokines, or a state of *i*NKT-cell anergy or depletion (106). This finding strongly suggests administration of lipid-based agonists or antagonists of *i*NKT and other lipid-specific T cells might be an alternative therapeutic approach to rhinitis and asthma in humans. All these new treatments must focus on providing a long-term cure and increasing patient compliance, if they are to warrant widespread application and replace symptomatic control of allergic diseases as exercised by current drugs (107).

Conclusions

As 'protein-only' allergens neither explain nor control the allergy march, we hypothesized that interactions of diverse pollen membrane biochemical compounds with particular APCs and specific T cells play distinct, but complementary, roles in allergic inflammation. The cypress pollen sensitivity model is useful to promote discussion about this mechanism underlying the complex emergence of allergy in a nonatopic adult population. We showed that different protein and nonprotein substances on the pollen surface interacted with several effector and regulatory T-cells, APC subpopulations and cytokines in the allergic mucosal milieu. Much more work is needed to characterize completely the fine antigen specificity of resident mucosal T-cell clones using natural, well characterized phospho- and glycol-lipids from pollens and an extensive panel of related synthetic lipid antigens. Recombinant protein allergens conjugated with DNA immunostimulatory sequences should be used in large-scale studies to achieve the complete safety profile of this new clinical approach. These investigations will provide insights into which T-cell population(s), among those responding to lipid or protein antigen stimulation at the mucosal surfaces, predominate, and trigger or perpetuate AHR and establish the best way to counteract their role in allergic disease.

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