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Vuoi evitare errori su farmaci e prodotti salutistici?

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Th22 cells in autoimmunity: a review of current knowledge

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Summary

Newly identified T helper cell 22 (Th22) is a subset of CD4⁺ T cells with specific properties apart from other known CD4⁺ T cell subsets. Th22 is obviously discrete from Th17 and Th1 subsets by production of interleukin (IL)-22 but not IL-17 or IFN-γ, and also with distinguished expression of aryl hydrocarbon receptor (AHR) as the key transcription factor. This T helper subset, by producing pro-inflammatory cytokines such as IL-22 and tumor necrosis factor-α (TNF-α), is implicated in the pathogenesis of inflammatory and autoimmune disorders. This review discusses the role of Th22 and its cytokine IL-22 in the immunopathogenesis of autoimmune disease including acute coronary syndrome, psoriasis, atopic dermatitis, rheumatoid arthritis, systemic lupus erythematosus, Behçet’s disease, type 1 and 2 diabetes and immune thrombocytopenia.

Introduction

Autoimmunity is the term for the immune conditions characterized by a specific response of immune system to the body’s self-tissues. Autoimmune diseases are the third largest category of illness in the industrialized world, following cardiovascular diseases and cancers. There are more than 80 types of autoimmune disorders which occur when self-immune system attacks and destroy healthy body tissue by mistake (1). Autoimmunity results from a break in self-tolerance involving humoral and/or cell-mediated immune mechanisms. The exact cause of self-tolerance breakdown is unknown, however, a variety of mechanisms have been suggested as the means by which self-tolerance is failure and autoimmunity occur. One mechanism is molecular mimicry, where a foreign antigen shares sequence or structural similarities with self-antigens. In this mechanism, some microorganisms or drugs may trigger changes that confuse the immune system. Molecular mimicry has typically been characterized on an antibody or T cell level. Accordingly, in a very general sense, with respect to the underlying mechanism, autoimmune diseases are divided into humoral and cell mediated autoimmunity (2-5). Both B and T cells can be made tolerant, however it is more important to tolerate T cells than B cells because B cells cannot make antibodies to most antigens without the help of CD4⁺ T cells. There is evidence that the classes and subclasses of cellular arms of immune system are implicated in autoimmunity. One of the main cells of immune system that is implicated in autoimmune diseases is CD4⁺ T helper (Th) cell. CD4⁺ T cells sub-divide conforming to the pattern of cytokines secretion (6). The naïve CD4⁺ T cells can differentiate into one of several subclasses, including Th1, Th2, Th3, Th9, Th17, and Th22, which produce different cytokines and chemokines to promote a specific type of immune response (7). Previously, Th1 cells were thought to be the main effector T cells responsible for the autoimmunity and inflammation.
Th22 cells in autoimmunity: a review of current knowledge

Furthermore, Duhen et al. have noticed which Th22 cells expressed these transcription factors very low or undetectable (12). It has been discovered that a distinct transcription factor called the aryl hydrocarbon receptor (AHR) mediated development of Th22 cells (13). In addition, study showed which stimulated AHR could contribute to production of IL-22 by notch signaling. In other words, notch-associated activation of CD4+ T cells result in elevation of IL-22 secretion even without induction of signal transducer and activator of transcription 3 (STAT3) (14). Indeed, these data indicate which Th22 cells are as a distinct lineage from the Th17, Th1 and Th2 subtypes (15, 16). It has been discovered which IL-6 and TNF-α could differentiate naive CD4+ T cells to Th22 cells, and on the other hand, this differentiation could be prevented by elevating concentrations of TGF-β (17). In addition, it has been reported which of conventional dendritic cells (DCs) and/or plasmacytoid DCs might lead to differentiation of naive CD4+ T cells to Th22 cells. By using activated conventional DCs and plasmacytoid DCs, Trifari et al. have revealed

**Figure 1** - The mechanisms of action of IL22. IL-22 produced by innate and adaptive immune cells especially Th22 is beneficial to the host defense in many infectious diseases. Moreover, depending on the target tissue, IL-22 could also be pathogenic in autoimmune disorder due to its inherent pro-inflammatory properties which are further enhanced when IL-22 is secreted together with other pro-inflammatory cytokines in particular IL-17. AMPs: anti-microbial peptides, RA: rheumatoid arthritis, SLE: systemic lupus erythematosus, ACS: acute coronary syndrome, AD: atopic dermatitis, T1D: type 1 diabetes, T2D: type 2 diabetes, ITP: immune thrombocytopenia.

![Diagram of IL-22 mechanisms](image)
that human DCs actually induce the development of Th22 cells from naive CD4+ T cells. Comparatively, plasmacytoid DCs by producing TNF-α and IL-6 are stronger than conventional DCs in the development of Th22 cells (10). IL-22 receptor is not expressed in cells of immune system. Thus, although IL-22 secreted by Th22 does not serve the communication between immune cells, however IL-22 is as a Th22 cell mediator which directly increase the innate, nonspecific immunity on epithelial and stromal cells including intestinal and respiratory epithelial cells, skin keratinocyte, hepatocytes, colonic subepithelial myofibroblasts, pancreatic acinar cells, and synovial fibroblasts derived from patients with rheumatoid arthritis (12, 18). On the one hand, Th22 and IL-22 play vital role against several infectious diseases, on the other hand, it might be pathogenic because of its inherent pro-inflammatory features; it would further elevated when IL-22 is produced together with other pro-inflammatory cytokines, in particular IL-17 (19). In recent years, multiple studies have demonstrated role of Th22 in many inflammatory and autoimmune disorders such as psoriasis (20), atopic dermatitis (21), rheumatoid arthritis (22), systemic lupus erythematosus (23), acute coronary syndrome (24), Behcet’s disease (25), ankylosing spondylitis (22), type 1 and 2 diabetes (26, 27), and immune thrombocytopenia (28).

**The role of Th22 in autoimmune disorder**

**Acute coronary syndrome**

Acute coronary syndrome (ACS) refers to any group of symptoms attributed to obstruction of the coronary arteries. Recent evidence has indicated that atherosclerosis is a chronic inflammatory disease with macrophages and T cells playing a critical role (29). CD4+ Th cells play main roles in the inflammatory process of atherosclerosis and also in the onset of ACS including unstable angina pectoris and acute myocardial infarction (30). It is demonstrated that ACS occurs as a consequence of coronary plaque rupture or plaque erosion, and changes in the functions of CD4+ T cells, especially Th cells, were found in patients with ACS (31). Among T cells, Th1 are recognized as having a pro-atherogenic role. In addition, Th17 and Th9 have been identified in atherosclerotic lesions. They have been linked to atheroma development by production of pro-inflammatory cytokines present in these lesions (24). Recently, Th22 have been identified in the atheromatous environment, and their presence and function has been investigated (32). Huang et al. suggest that Th22 cell is a gradually proved potential biomarker for ACS. In a study, Oliveira et al. showed the presence of Th17, Th2, and Th22 in human carotid lesions and indicate that interactions among them may contribute to the atheroma progression and destabilization (33). In another research, Lin et al. in 2013 indicate that AHR expression, peripheral Th22 number and their effector cytokine IL-22 levels were obviously increased in patients with acute myocardial infarction, stable and unstable angina pectoris compared with patients without coronary artery disease, indicating that peripheral Th22 cells played major roles at the ACS (32). Interestingly, similar data was evident by Zhang et al., which demonstrated that the Th22, Th17 and Th17/Th1 cells were considerably higher in acute myocardial infarction and unstable angina pectoris patients than those of stable angina patients and healthy control (34). In an experimental model Hanawa et al. reported that IL-22 could interact with fibroblasts, smooth muscle cells, and endothelial cells in the rat experimental autoimmune myocarditis (35). Moreover, there was a positive correlation between the frequency of Th22 cells and IL-22 concentration in acute myocardial infarction and unstable angina pectoris patients (34). In a study, Xia et al. showed that Th22 cells played pivotal roles in coronary plaque rupture or plaque erosion, since IL-22 was detected in the carotid plaque (36). These findings confirmed increased frequencies of IL-22, Th22 and Th17 cells in ACS patients, which showed that Th22 and Th17 cells may play a potential role in plaque destabilization and the development of ACS (34).

**Psoriasis**

Psoriasis is a common chronic inflammatory disorder which is identified by red scaly skin plaques with hyperproliferative keratinocytes. Epidermal hyperplasia and infiltration of inflammatory cells into skin lesions are major histological results in patients with psoriasis (37, 38). A key event in psoriasis is migration of immune cells from the dermis into the epidermis, where they induce keratinocytes proliferation (38). Although pathogenesis of psoriasis is unknown, however it has been identified which several immune cells including DCs, CD4+ T cell subsets (Th17, Th22, and Th1 cells), CD8+ T cells and neutrophils exist in psoriatic skin lesions and might involve in etiology of psoriasis disorder (38). IL-17 and IL-22 are synthesized by Th17, Th22, and Th1 in psoriatic skin lesions (38). It has supposed that Th1 is principally correlated with the pathogenesis of psoriasis, but Kagami et al. suggested which Th17 cells trough secretion of IL-17 and IL-22 are more implicated. They also reported which there was an augmentation of Th22 and Th1 cells in psoriatic patients, but to a lesser degree (38). Conversely, Fujita et al. suggested that the majority of IL-22-producing CD4+ T cells are neither Th17, Th1, and nor Th2 however; they did not study the majority of Th22 in psoriasis. Indeed, psoriasis skin lesions contain a population of T cells that co-synthesize IL-17 and IL-22, but the majority of IL-22-producing T cells is neither Th17, Th1, nor Th2, and may represent a unique subset of IL-22-producing helper T cells, Th22 (39). Eyerich et al. have reported that although Th22 similar to Th17 are scarcely detected...
in PBMCs, but Th22 cells are largely founded in T-cell population isolated from the skin of psoriasis patients (15). Moreover, it has been discovered which Th22 clones derived from psoriatic patients are constant in vitro and demonstrate a transcriptome profile apparently different from Th1, Th2, and Th17 cells. Therefore, one supposes that genes encoding proteins including Fibroblast growth factors (FGFs) and chemokines might contribute to angiogenesis and fibrosis. It has been noticed that when primary human keratinocytes cultured with Th22 supernatants, it led to expression of transcriptome response profile which comprised genes involved in adaptive and innate immunity through activation of T cell and NK promoting factors including IL-15 and IL-7, in keratinocytes. In addition, IL-32 (as a TNF-α-enhancing cytokine) is produced by keratinocytes and result in production of TNF-α from Th22 cells, which might activate pro-inflammatory Th22 responses. Then, there is a synergistic dependency between the pro-inflammatory responses of Th22 and IL-22 and TNF-α (15, 40). Conversely, it has been shown an elevated wound healing in an in vitro injury model Th22 supernatants that exclusively related to IL-22 (15). It is suggested, IL-22 alone could play an important role in expression of multiple genes which contribute to tissue repairing and wound healing including kallikerin subgroup of serine proteases and serpin family of protease inhibitors (41, 42).

Atopic dermatitis

Atopic dermatitis as a chronic inflammatory skin disease identified by episodes of acute eczema alternating and cutaneous hyperreactivity to environmental triggers and often is seen in patients with personal or family history of asthma and allergic rhinitis (43, 44). It is considered that expansion of atopic dermatitis belongs to disease-specific and time-dependent recruitment of various leukocytes could influencing resident skin cells by cytotoxic mechanisms (45). It is supposed in the past which Th2 cells are responsible for atopic dermatitis diseases, but nowadays Th17 and Th22 cells have considered which involve in development of atopic dermatitis disease (45, 46). Indeed, Th17 and Th22 cells specifically involve in dialogue with non-immune cells. In this case, role of Th17 and Th22 cells in multiple immune–associated skin disorder including psoriasis, atopic dermatitis, and allergic contact dermatitis is defined (45). Koga et al. have demonstrated an elevation of percentage of Th17 cells in peripheral blood of patients with atopic dermatitis which has correlated with severity of disease (47). In contrast, Nograles et al. have founded an elevation of production of IL-22 from T22 cells in lesional skin of patients with atopic dermatitis. They also have noticed a significant increase in population of Th1 and Th17 cells in psoriatic skin in comparison with atopic dermatitis, while population of Th2 and Th22 were strongly increased in atopic dermatitis (46). Furthermore, Nograles et al. have reported a significant augmentation of IL-22-producing T-cell cells in atopic dermatitis patients compared with psoriasis. In this study, they have seen an extreme number of CD4+ and CD8+ cells which could uniquely produce IL-22. Indeed, these cells were responsible for almost 70% of the IL-22 secretion in atopic dermatitis disease, with low frequencies of Th1, Th2, and Th17 T-cells that co-secrete IL-22 (46). Overall, these data indicate that the new subsets of IL-22 producing Th22 and Tc22 T cells could involve in elevated expression of IL-22 in chronic atopic dermatitis skin, and contribute to Th2/T22 immune polarization in patients with chronic atopic dermatitis.

Rheumatoid arthritis

RA is considered as a chronic inflammatory disease that identified by the reposition and proliferation of inflammatory cells in the synovial (joint) space. According to RA is a chronic disease, inflammation of several joints leads to damage of the joint cartilage and ablation of bone. Pathogenesis of RA is unclear yet, however it is observed the activation of multiple cells such as T cells, B cells, macrophages, mast cells, and fibroblast-like synoviocytes (FLSs) which involve in synovial inflammation and joint destruction (48). CD4+ T helper cells contribute to the development and progression of RA. Among CD4+ T helper cells, recognition of Th17 cells resulted in better understanding of pathogenesis of RA (48). Although it recently has been demonstrated Th22 cells involve in the pathogenesis of RA, however its role in the pathophysiology of RA still has remained undefined. For the first time, Zhang L et al. has been identified which augmentation of Th22 cells could associated with Th17 cells in RA patients. They have been demonstrated that Th22 and Th17 cells as well as IL-22 were significantly increased in RA patients in comparison with osteoarthritis and healthy individuals; however it has not observed significant difference regarding Th1 cells and IL-17. Furthermore, this study has been reported a positive correlation regarding Th22 cells with IL-22 and Th17 cells in patients with RA (49). Later, another study evaluated frequencies of Th22 cells, Th17 cells and Th1 cells in both RA patients and anklyosing spondylitis. Again, it has been reported elevation of Th22 cells, Th17 cells and IL-22 in patients with RA and anklyosing spondylitis in comparison with osteoarthritis patients and healthy individuals. Furthermore, consistent with previous study, it has been reported Th22 has a positive correlation with Th17 cells and IL-22. However, although it has shown that the frequency of Th22 and Th17 cells were positively correlated with disease activity in RA patients, but this correlation has not seen in anklyosing spondylitis patients (22). Van Hamburg et al. have demonstrated elevation of Th17 and Th22 cell populations in patients with RA similar to previous studies, which were existed in RA synovial fluid. Moreover, they have found that Th17 were more potent to
stimulate synovial fibroblasts (RASF) in production of IL-6, IL-8, MMP-1 and MMP-3 compared with Th22 cells. These data uncover which formation of synovial inflammation by IL-17A/Th17 cell is independent of Th22 cells and IL-22 (50). However, Zhao et al. have found which percentages of Th22 cells in RA patients correlated positively with the levels of plasma IL-22 (51), but a positive correlation between plasma IL-22 and Th17 cells were seen only in ankylosing spondylitis patients not in RA patients (22).

**Psoriatic arthritis**

Psoriatic arthritis is a joint disease characterized by both psoriasis and a related form of inflammatory arthritis (52). Increased frequencies of Th17 and Th22 cells along with their pro-inflammatory cytokine network, including TNF-α, IL-17, and IL-22, are the feature of both skin lesions (plaques) in psoriasis and synovium in psoriatic arthritis. However, their different distribution at disease tissues, including lower frequencies of IL-22+CD4+ T cells in synovial fluid compared to skin and peripheral blood, and lack of IL-22 expression in synovial tissue indicate that Th17 and Th22 cells, have a common and joint role as well as divergent roles in the pathogenesis of psoriasis and psoriatic arthritis. Benham et al. demonstrate increased frequencies of Th17 cells in peripheral blood of patients with psoriasis and psoriatic arthritis. Their findings showed that IL-17 secretion was remarkably elevated in both psoriasis and psoriatic arthritis, whilst IL-22 secretion was higher in psoriatic arthritis compared to psoriasis and healthy controls (53, 54). In patients with psoriatic arthritis, Th17 cells number were elevated in synovial fluid compared to peripheral blood. Moreover, increased frequencies of IL-17+ and IL-22+ CD4+ T cells were demonstrated in psoriasis skin lesions. In contrast, the increased frequency of Th17 cells was seen in psoriatic arthritis synovial fluid compared to peripheral blood, whereas as frequency of Th22 cell was lower. In conclusion, when IL-17 expression is equal in psoriatic arthritis, osteoarthritis and RA synovial tissue, IL-22 expression was higher in RA than either osteoarthritis or psoriatic arthritis synovial tissue, in which IL-22 was remarkably absent (53).

**Diabetes**

Among autoimmune diseases, type 1 diabetes (T1D), also named autoimmune diabetes, have afflicted 10 million peoples worldwide. This disease is caused by autoimmune-mediated destruction of pancreatic-cells, leading to insulin deficiency, hyperglycemia and complications. Many components of the immune system are implicated in autoimmune leading to β cell destruction, including cytotoxic and helper T-cells, B-cells, macrophages, and DCs (55). Cytokines produced by these cells have also been shown to play a key role in β cell destruction and regulation of autoimmunity in T1D. The inflammatory process in early diabetes is thought to be initiated and propagated by the effect of Th1-secreted cytokines e.g. IFN-γ (55). It is showed that levels of IL-6 and TNF-α may be useful in the prediction of proliferative diabetic retinopathy, whereas higher IL-10 levels are related to lower risk of diabetic retinopathy in diabetes patients (56). Dalmas et al. showed a pronounced pro-inflammatory signature of adipose tissue macrophages in type 2 diabetic (T2D) obese patients, frequently driven by increased NLRP3-dependent IL-1β production. It is revealed that IL-22 increased IL-1β release by inducing pro-IL-1β transcription via activation of C-Jun pathways in macrophages. These findings identified IL-1β and IL-22 as main players in adipose tissue inflammation, with a pathological relevance to obesity-induced T2D (57). However, Chenet al. found that the mean IL-22 serum levels were somewhat lower in diabetic patients than in normal controls (58). It is known that IL-22 can up-regulate Regenerating (Reg) genes expression in islets and could potentially induce regeneration of β cells and inhibit their apoptosis. Finally, cytokines both induce and regulate inflammatory condition and have the potential to regenerate and preserve insulin-producing β cells in the islets (59). In a study by Xu et al. Th17 and Th22 were significantly elevated in patients with T1D compared to control donor, while there were no significant differences in Th1 cells. Also, Th22 cells showed a positive correlation with Th17 cells in these patients. However, there was not any correlation between IL-17 and IL-22 in sera. Therefore, these findings showed that Th22 may contribute to the pathogenesis of T1D (27).

The systemic chronic inflammation has been postulated to bridge the increased risk of cardiovascular disease and T2D. In newest study Zhao et al. suggest that both peripheral frequencies and total numbers of Th1, Th17, and Th22 cells were further increased in diabetic patients with coronary atherosclerotic heart disease. Further analysis confirmed that increased pro-inflammatory Th cells, especially Th22, were independent risk factors of cardiovascular complication in diabetes. Furthermore, Th1 and Th22 frequency demonstrated considerable potential in predicting coronary atherosclerotic heart disease in diabetes (60). In another study Zhao et al. showed an increased Th22 frequencies and IL-22 concentrations in obesity and T2D (26). Some data indicate a conceivable role of Th22 cells in diabetic retinopathy. Although, Chenet al. suggested that IL-22 serum levels were slightly lower in diabetic patients than in normal controls but the IL-22 level of PBMCs was clearly elevate in patients with proliferative diabetic retinopathy compared with the level in patients with non-proliferative diabetic retinopathy and healthy controls (58). Finally, the significant correlation of mentioned data implied that Th22 might play a more important role in both insulin resistance and β-cell impairment.
Behçet’s disease

Behçet’s disease (BD) as a recurrent systemic inflammatory disease is identified by oral and genital mucous ulcer, intraocular inflammation (uveitis) and skin lesions (61). It has been reported which diminution of Tregs cells and increase in population of Th1 and Th22 cells as well as Th17/Th1 cells could involve in pathogenesis of BD (62). Aktas et al. have uncover which population of Th1 and Th22 cells have strongly elevated, and percentage of Treg cells have dramatically decreased in patients with Behçet. Moreover, they have shown which the frequency of recurrent oral ulcers was associated with elevation of Th22 cells in patients with Behçet (62). IL-22 (as a major cytokine of Th22) is correlated with disease activity as well as presence of small vessel inflammation in Behçet’s disorder which could clarify the role of IL-22 in pathogenesis of this disease (63). It has been founded that uveitis is major reason of vision loss in BD which manifested due to recurrent ocular inflammatory attacks (64). It is supposed that IL-22 producing CD4+ T cells trough reaction with self-antigens might involve in pathogenesis of uveitis (25). Cai et al. have recognized an increase in expression level of IL-22 in supernatants of stimulated PBMCs and CD4+T cells of BD with active uveitis compared with BD without active uveitis and healthy individuals. Furthermore, they have identified an elevation of IL-22-producing CD4+ T cells population in BD patients with active uveitis (63). Sugita et al. have confirmed above data and founded Th22 clones from ocular samples taken from BD secreted high amounts of IL-22 and TNF-α cytokines, but not IFN-γ and IL-17. Also, they have demonstrated which CD4+ T cells related to BD patients in the presence of IL-6 and TNF-α in vitro could differentiated into Th22 cells and polarized Th22 cell could secret a large amount of IL-22. In addition, IL-22-producing T cells in the presence of retinal antigens could produce high level of IL-22 in mice with experimental autoimmune uveitis. These data indicate which IL-22 and TNF-α producing Th22 cells probably involve in ocular immune response in BD patients (25).

Immune thrombocytopenia

Immune thrombocytopenia (ITP) is an autoimmune disorder characterized by low count platelet due to decreased platelet production as well as increased platelet destruction by autoimmune mechanisms in which the patient’s immune system reacts with a platelet of autoantigens. In ITP a shift toward B cells producing autoantibodies together with CD4+ T helper cells has been reported. Cao et al. suggested that the plasma IL-22 levels in ITP patients were significantly higher than that in healthy controls, and this elevation was correlated with Th1 and Th22 cells in these patients (65). In addition, it is shown that the percentages of both Th1 and Th22 cells in ITP patients elevated as compared to healthy controls. Whereas, the percentage of Th17 cells was not significantly different between ITP patients and control groups, and there was no statistical correlation between the IL-22 level and the percentage of Th17 cells in active ITP patients. Therefore, the elevated IL-22 level correlates to Th1 and Th22 cells percentage, which may play a synergistic effect in the immunopathogenesis of ITP, while Th17 cells may not be correlated with the occurrence of active ITP (66). In contrast, Wu et al. in their recent study showed that the proportion of peripheral blood Th1, Th17, Th22 cell subgroups and the levels of IFN-γ, IL-17, IL-22 in culture supernatant increased in chronic ITP patients (67). In another study by Hu et al. Th22 cells showed a positive correlation with the levels of plasma IL-22 as well as Th17 and Th1 cells in ITP patients. Additionally, the proportion of Th22 cells was higher in autoantibody-negative ITP patients than in autoantibody-positive patients (68). To investigate the change of Th22 cells in the peripheral blood of the primary ITP patients and evaluate the significance of Th22 cells in ITP, Liu et al. used the peripheral blood of ITP before and after therapy, in ITP patients. The results indicate that proportion of Th22 cells and the levels of cytokine IL-22, IL-6, TNF-α, and IL-22 mRNA in patients before and after therapy were significantly higher than those in healthy group. Briefly, in ITP patients, the number of Th22 cells and the levels of TNF-α and IL-6 increase, whereas the level of TGF-β decreases (28). In a clinical trial, Cao et al. evaluated the effects of dexamethasone on regulating IL-22 production and correcting Th1 and Th22 polarization in ITP. In this study plasma IL-22 concentration and the proportion of Th1 and Th22 cells were significantly increased in pretherapy patients compared to healthy controls, whereas, high-dose dexamethasone administration reduced IL-22 production and also corrected the imbalance between Th1 and Th22 subsets. They concluded that IL-22 levels were positively associated with Th1 and Th22 cells in ITP patients before and after dexamethasone therapy (69).

Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is an autoimmune disease that identified by acute and chronic inflammation of several tissues such as skin, kidneys, joints, brain, and other organs. Several abnormalities in activated immune system of patients with SLE including secretion of autoantibodies, defective elimination of autoantibodies, complement and cytokine activation, accumulation of immune-complex in tissue involve in tissue and organ damages. Hence, defective immune tolerance against self-antigens as well as extensive T cells and B cells activation contribute to development of SLE (70). IL-17 and IL-22 which are mainly secreted by Th17 and Th22 cells respectively, could clarify progress and induction of autoimmune phenomena (71, 72). Zhao et al. have recognized which
the population of Th1, Th17 and Th22 cells as well plasma IL-22 and IL-17A were strongly increased in patients with SLE in comparison with control individuals. They also evaluated the frequency of Th22 and Th17 cells in these patients and founded which although there is a positive correlation between Th22 and Th17 cells, however this correlation was not seen with the other inflammatory values including CRP, ERS and C3 in SLE patients. These data regarding Th17 and Th22 may shed a new light into the way toward the better understanding of role these cells in pathogenesis of SLE disorder (72). In recent years, role of Th22 cells and IL-22 in pathogenesis of SLE have been more identified. In one study, it has been noticed which reduced IL-22 levels, but not elevated IL-17 and IL-23 levels, were associated with disease activity in SLE patients (73). However, Yang et al. offered which although Th17 could associate with activity of SLE, however Th22 but not Th17 might consider as a good index to predict the tissue involvement of SLE. The major results is concentrated on elevation of Th22 cells and serum IL-22 levels in patients with sole lupus skin disease and reduction of Th22 cells and serum IL-22 levels in patients with sole lupus nephritis. Moreover, it has been discovered a positive correlation between Th22 cells but not Th1 and Th17 cells with IL-22 secretion and plasma IL-22 levels (23, 74). In a novel study by Lin et al. founded which correlation between plasma IL-22 levels and Th22 cells could different features in new-onset of patients with SLE than relapsing SLE patients. It has been noticed that there was a significant reduction of the IL-22 levels in new-onset SLE patients in comparison with relapsing SLE patients and healthy individuals (74).

Conclusion

In autoimmunity, one of the most important players is the CD4 T cell. The CD4 T cell lineage consists of a number of phenotypically and functionally distinct subsets. Recently, Th22 cells were identified as a Th cells subset that produce IL-22 and TNF-α and are distinct from Th1, Th2, and Th17 cells. Th22 and its cytokine IL-22 are implicated in the immunopathogenesis of autoimmune diseases; therefore, therapeutic approach based on the pharmacological signalling disruption of IL-22 could be useful for the treatment of these types of diseases (75). Treatment with recombinant cytokine or gene therapy for IL-22 may reduce tissue destruction during inflammatory responses. It is demonstrated that in the presence of anti-TNF-α- and anti-IL-6-blocking antibody, Th22 cells failed to produce IL-22. In addition, infliximab-pretreated Th22 cells produced less IL-22 and TNF-α (25). In a study, Mitra et al. demonstrated successful inhibition of IL-22 induced fibroblast like synoviocytes proliferation by anti-IL-22R antibody with blocking of IL-22/IL-22R interaction, which may be considered as a novel therapeutic target for psoriatic arthritis (54). However, others believe that targeting IL-22 or Th22 might provide pathogenic treatment because in one side it is difficult to generalize whether Th22 cell is protective versus pathogenic. On the other side, IL-22 function could not entirely reflect Th22 function, since IL-22 apart from Th22 cells is also expressed by other cells. Hence, targeting Th22 or IL-22 is nonselective and may affect all of the Th22 and IL-22 in the whole body, leading adverse side effects. However, it is suggested that the restricted expression of IL-22R1 in non-lymphoid cells could lead to a decrease of side effects mediated by immune responses (75). Therefore, further studies are required for clarifying the accurate role of Th22 and IL-22 in autoimmunity.

References

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Cross-reactivity of a new food ingredient, dun pea, with legumes, and risk of anaphylaxis in legume allergic children

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\textsuperscript{2}Internal Medicine, Clinical Immunology and Allergology, Nancy University Hospital, France
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\textbf{Key words}
Cross-reactivity; dun pea; legume allergy; peanut allergy; specific IgE

\textbf{Summary}
Background. Legume allergy is the fifth food allergy in Europe. The dun pea (\textit{Pisum sativum sativum var. arvense}), a pea belonging to the same subspecies as green pea, has been recently introduced as an ingredient in the human food industry. The aims of this study were to evaluate the cross-reactivity between dun pea and other legumes and to search for modification of allergenicity induced by food technologies. \textbf{Methods.} A series of 36 patients with legume and/or peanut allergy was studied. They underwent skin tests to peanut and a panel of legumes including dun pea. Specific IgE to dun pea and cross-reactivity to peanut allergens, particularly to Ara h 1, were evaluated by ELISA. Proteins and allergens of different pea extracts were studied by SDS-PAGE and immunoblots. \textbf{Results.} In France and Belgium, 7.7\% of severe food anaphylaxis cases were due to legumes. Patients with isolated legume allergy had positive prick tests to dun pea, whereas patients with isolated peanut allergy had negative prick tests. Cross-reactivity between sIgE to peanut and dun pea was observed, and more frequently than expected (96\%) peanut-allergic patients with legume sensitization or allergy had sIgE to Ara h 1. Analysis of dun pea allergens suggested that protein epitopes were presented differently in dun pea seeds, isolate and flour. \textbf{Conclusions.} This study identifies, for the first time, a risk of dun pea allergy in legume-allergic patients and in a subset of peanut-allergic patients.

\textbf{Introduction}
Legumes are a staple food in many European and Asian countries. Common edible seeds are soybean, lentil, chickpea, green pea, white bean, the spice fenugreek, and lupine seed flour used as an ingredient. Although the peanut belongs to the \textit{Leguminosae} family, it represented a particular case because peanut allergy is more often isolate, without clinical cross-reaction with pre-cited legumes. Legume allergy is the fifth most prevalent food allergy in Spanish children (1). In France, the records from the Allergy Vigilance Network point for the period 2002-2012 (\textbf{table 1}) to a prevalence of 6.8\% out of 566 children (4\textsuperscript{th} rank after peanut, tree nuts and milk) and 8.3\% out of 684 adults (3\textsuperscript{rd} rank after shellfish and tree nuts) (2). Sensitivity to legumes is frequent in Japan for soybean, in India for chick pea (3) and to a lesser degree in the USA (4). There is now genuine concern about sensitivity to legumes with the extensive use of protein ingredients in industrial foods. Given the public health threat related to GMOs, concentrates and isolates of soy proteins are often replaced by lupine flour. The growing incidence of anaphylaxis to lupine proteins, including a specific risk for peanut-allergic patients, has been demonstrated (5-7). In 2006, lupine and derivative products were added to the list of allergenic foods requiring mandatory labelling in the European Union (8). Consequently, food manufacturers have increasingly used another source of legume protein, botanically very close to green pea, dun pea (\textit{Pisum sativum sativum var. arvense}). It was originally cultivated for animal food. Currently, dun pea proteins are found in the breadcrumbs used for coating meats, in the ingredients of minced steak, in specialized food for sportsmen and in pharmaceutical protein substitutes. In the absence of mandato-
Cross-reactivity of a new food ingredient, dun pea, with legumes, and risk of anaphylaxis in legume allergic children

Group 1 included 6 patients with isolated legume allergy. In this group, peanut allergy was excluded because of a negative history, negative PT and the absence of specific IgE (sIgE) to Ara h 2, 3, 6 and 7 (9).

Group 2 included 30 patients with peanut allergy: (i) Subgroup 2a: 13 patients with isolated peanut allergy and not sensitized to legume or with current consumption of legumes without any clinical reaction, (ii) Subgroup 2b: 8 patients sensitized to legume (on avoidance diets for legumes, without any previous clinical reaction), and (iii) Subgroup 2c: 9 patients with both peanut and legume allergies.

Skin testing

PTs were performed in accordance with previously published methodology (10). PT was considered positive if the mean wheal diameter was at least 2.5 mm larger than the diameter of the negative control. The positive control was codeine phosphate 9% (ALK-Abello, France). The timing of recording was 15 minutes. Fresh raw legumes were tested: green pea, chickpea, lentil, soybean, white bean, broad bean, and roasted peanut. Dun pea was tested using protein isolate, Pisane® M9 (Cosucra, Belgium) and lupine (Lupinus albus) as a flour (Sotexpro, France).

Specific IgE measurements and inhibitions

Since Ara h 1 shares a 50% homology with the green pea allergen Pis s 1, sIgE to rAra h 1 were measured by enzyme-linked immunosorbent assay (ELISA). Recombinant Ara h 1 (9) was coated to microplate wells (MaxiSorp®, Nunc). After blocking, the diluted serum 1:100 was incubated for two hours. The presence of IgE was revealed by addition of horseradish-peroxidase (HRP) labeled goat anti-human IgE (KPL, MN, USA) and substrate UltraTMB (Sigma). Specific IgE were extrapolated compared to a standard curve using purified IgE (Millipore, CA, USA) and final results.

Table 1 - 96 cases of severe anaphylaxis to legumes registered between 2002-2012 by the French Allergy Vigilance Network (diagnosis established on an anaphylactic reaction (grade 2, 3 and 4) and further work-up showing positive prick test and specific IgE).

<table>
<thead>
<tr>
<th>Legumes</th>
<th>Children ≤ 16 years</th>
<th>Adults &gt; 16 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean</td>
<td>15</td>
<td>21</td>
</tr>
<tr>
<td>Lupine flour</td>
<td>7</td>
<td>34</td>
</tr>
<tr>
<td>Lentil</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Green pea</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Dun pea</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Chickpea</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Fenugreek</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>White bean</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Broad bean</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Lucerne (alfalfa)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total / total cases of FA</td>
<td>39/566   6.8%</td>
<td>57/684  8.3%</td>
</tr>
</tbody>
</table>

FA: food allergy

Material and Methods

Patients

The study was approved by the local ethical committee and written informed consent was obtained from the parents of each subject, which allowed the use of the samples for research purposes (authorization No. AC-2008-449 of French Ministry of Research). Thirty-six patients were recruited. The clinical criteria of selection were isolated or associated clinical allergy to legumes or peanut. Twenty-nine patients had prick tests (PT) to a legume panel including dun pea. The seven remaining patients were not tested because of current consumption of legumes without any clinical reaction.

ry labelling, dun pea is a masked ingredient under the generic term of vegetal protein. The ingredient used is a concentrate or an isolate of the protein delivering a large amount of protein. Moreover, the commercial advertising from the manufacturers of this ingredient guarantees that it is a non-allergenic food. Since several cases of severe anaphylaxis to dun pea have been registered by the French Allergy Vigilance network, the aims of this study were to evaluate the rate of sensitization to dun pea in legume-allergic patients and in peanut-allergic patients, and to search for modification of allergenicity induced by food technologies.
were expressed in kU/L. Values were means of three wells. Specific IgE > 1.0 kU/L were considered positive. For ELISA inhibition, the immuno-assay was performed as explained above except that dun pea isolate extract was coated to microplate wells, and the diluted sera pre-incubated overnight with peanut extract. Results are expressed as inhibition percentage.

**SDS-PAGE, Immunoblot and immunoblot inhibition assays**

Proteins of pea extracts (13 µg) were separated by SDS-PAGE and revealed by Coomassie blue staining or transferred to polyvinylidene difluoride (PVDF) membrane (0.45 µm, GE Healthcare, Buckinghamshire, UK) for immunoblotting. After blocking, membranes were incubated with patient’s serum diluted 1:5 in TBST buffer (100 mM Tris pH 7.5, 154 mM NaCl, 0.1% (v/v) Tween 20) containing 5% (w/v) defatted milk (TBSTM). Membranes were then washed with TBST buffer and incubated with (HRP) labeled polyclonal anti-human IgE (dilution 1:1000 in TBSTM). After washing, IgE-reactive bands were revealed by chemiluminescence (ECL Advance, GE Healthcare, Buckinghamshire, UK). Two Negatives controls immunoblots were carried out: first one with the anti-human IgE antibody alone, and second with a serum of a non-allergic patient. They were performed for all extracts (data not shown). Immunoblot inhibition assays were carried out using the same method, except that the sera were pre-incubated overnight at 4°C with 650 µg proteins (50x excess) of dun pea or dun pea flour extracts.

**Results**

**Cross-reactivity of dun pea with other legumes**

Thirty-six patients were recruited to investigate clinical and biological cross-reactivity between dun pea and other legumes. Group 1 (isolated legume allergy) included allergy to lentil (4), dun pea (3), green pea (3), soybean (2), broad bean (2), lupine (1), chickpea (1) (table 2). PTs were positive for at least four legumes. PT to dun pea was positive in 6/6 cases (6.5-23 mm; mean: 12.4 mm). Specific IgE to dun pea were present in 5/6 sera (0.5-83 kU/L).

**Table 2 - Group 1: Patients with isolated legume allergy.**

<table>
<thead>
<tr>
<th>ID</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Legumes</th>
<th>Symptoms</th>
<th>Time between first reaction and tests</th>
<th>PT to legume (mm)</th>
<th>sIgE</th>
<th>Inhibition of dun pea by peanut (%)</th>
<th>sIgE to Ara h 1 (kU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HH</td>
<td>F</td>
<td>1</td>
<td>soybean</td>
<td>AD</td>
<td>4 months</td>
<td>7.7</td>
<td>8</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>RY</td>
<td>M</td>
<td>8</td>
<td>green pea</td>
<td>LAO</td>
<td>3 years (positive LT grade 3)</td>
<td>12</td>
<td>5</td>
<td>6</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>dun pea</td>
<td>Localized U, Cj</td>
<td>1 year (positive LT grade 4)</td>
<td>12</td>
<td>5</td>
<td>6</td>
<td>2.3</td>
</tr>
<tr>
<td>SK</td>
<td>M</td>
<td>8</td>
<td>lentil</td>
<td>U, AO, Ab P</td>
<td>1 year</td>
<td>6.5</td>
<td>7.5</td>
<td>12</td>
<td>7 &lt; 1.0</td>
</tr>
<tr>
<td>HE</td>
<td>M</td>
<td>18</td>
<td>green pea</td>
<td>U, LAO</td>
<td>16 years (negative OC to 7 g)</td>
<td>23</td>
<td>7</td>
<td>12</td>
<td>6.85</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>soybean</td>
<td>U</td>
<td>16 years (negative OC to 7 g)</td>
<td>23</td>
<td>7</td>
<td>12</td>
<td>6.85</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>lentil</td>
<td>U</td>
<td>16 years (negative OC to 7 g)</td>
<td>23</td>
<td>7</td>
<td>12</td>
<td>6.85</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>chick pea</td>
<td>U</td>
<td>16 years (negative OC to 7 g)</td>
<td>23</td>
<td>7</td>
<td>12</td>
<td>6.85</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>broad bean</td>
<td>U, C</td>
<td>8 years (negative OC to 7 g)</td>
<td>23</td>
<td>7</td>
<td>12</td>
<td>6.85</td>
</tr>
<tr>
<td>PC</td>
<td>F</td>
<td>8</td>
<td>green pea</td>
<td>AD</td>
<td>7 years (positive OC to 60 g)</td>
<td>12</td>
<td>11</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>lentil</td>
<td>AD</td>
<td>6 years (positive LT grade 3)</td>
<td>12</td>
<td>11</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>broad bean</td>
<td>LAO, U</td>
<td>4 years (positive LT grade 2)</td>
<td>12</td>
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<td>9</td>
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<tr>
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<td></td>
<td></td>
<td>lentil</td>
<td>AO</td>
<td>3 years</td>
<td>13.5</td>
<td>6</td>
<td>9</td>
<td>&lt; 0.35 nd 4</td>
</tr>
</tbody>
</table>

Cross-reactivity of a new food ingredient, dun pea, with legumes, and risk of anaphylaxis in legume allergic children

Table 3 - Peanut-allergic subjects with isolated peanut allergy (Subgroup 2a) or with sensitization to legumes (Subgroup 2b).

<table>
<thead>
<tr>
<th>ID</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Sensitization / Avoidance of legumes</th>
<th>Peanut PT (mm)</th>
<th>Peanut sIgE (kU/L)</th>
<th>sIgE to dun pea (kU/L)</th>
<th>sIgE to green pea (kU/L)</th>
<th>sIgE to chick pea (kU/L)</th>
<th>sIgE to lentil (kU/L)</th>
<th>sIgE to lupine (kU/L)</th>
<th>sIgE to soybean (kU/L)</th>
<th>sIgE to white bean (kU/L)</th>
<th>sIgE to broad bean (kU/L)</th>
<th>sIgE to dun pea (kU/L)</th>
<th>sIgE to Ara h 1 (kU/L)</th>
<th>Inhibition of dun pea by peanut (%)</th>
<th>sIgE to Ara h 1 (kU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2a</td>
<td>VE</td>
<td>F</td>
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<td>5</td>
<td>45.6</td>
<td>3.5</td>
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<td>BS</td>
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<td>0.965</td>
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<td>nd</td>
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<tr>
<td></td>
<td>BR</td>
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<tr>
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<td>0</td>
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<td>0</td>
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</table>

S: sensitization, E: eviction, DBPCFC: double-blind placebo controlled food challenge, ED: eliciting dose

Patient HE had allergy to all legumes since infancy. The recent episode of urticaria and angioedema was linked to dun pea. PT was impressive: 23 mm and sIgE were 83 kU/L. Subgroup 2a (isolated peanut allergy) had negative PTs to all legumes including dun pea in 6/6 cases (table 3). They were not performed in seven cases since patients ate all legumes without any reaction. Specific IgE to dun pea were present in only one case out of 13 and at a low level (0.7 kU/L).

Subgroup 2b (peanut allergy and sensitization to legumes) was sensitized to between one and five legumes (table 3). PT to dun pea was positive in 4/7 patients. Specific IgE to dun pea were present in 3/8 sera.

Subgroup 2c (peanut and legume allergy) had allergy to green pea (4), dun pea (3), lentil (3), soybean (2) and lupine (1) (table 4). This group had positive PTs to between one and five legumes. PT to dun pea was positive in 9/9 cases (2 cases at 17 mm, mean: 8.8 mm). PTs to green pea and lentil were positive less often, in 2 and 4 cases, respectively. Specific IgE to dun pea were positive in 9/9 cases (0.8 - 68.8 kU/L).

Out of 15 cases of allergy to legumes (group 1 and subgroup 2c), 9 had peanut allergy (subgroup 2c). Conversely, out of 30 cases of peanut allergy, at least 9 also had legume allergy. However, 8 patients were on avoidance diets because of legume sensitization and we cannot be certain of tolerance in the event of ingestion.
very high: PTs were positive in the 6 patients with history of
dun pea allergy (3 cases in group 1 and 3 cases in group 2c). It
should be noted that in 5 green pea allergic-patients, PTs to
dun pea were positive in all patients, though PTs to green pea
were negative in some cases (patient PC in group 1 and patients
MM, ML, KQ and MB in group 2c).

Nineteen patients had positive PTs to dun pea and/or green pea.
The wheal diameter of PTs to dun pea (9.1 ± 5.6 mm) was sig-
nificantly higher than those of green pea (4.3 ± 4.3 mm) (p =
0.006). These observations could be related to the fact that dun

**Table 4 - Peanut-allergic subjects with legume allergy (Subgroup 2c).**

<table>
<thead>
<tr>
<th>ID</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Legume allergy</th>
<th>Symptoms</th>
<th>Time between first reaction and tests</th>
<th>PT to peanut (mm)</th>
<th>IgE to peanut (kU/L)</th>
<th>Positive DBPCFC to peanut: ED (g)</th>
<th>Dun pea</th>
<th>Green pea</th>
<th>Chick pea</th>
<th>Lentil</th>
<th>Lupine</th>
<th>Soybean</th>
<th>White bean</th>
<th>Broad bean</th>
<th>DBPCFC to dun pea: ED</th>
<th>sIgE to dun pea (kU/L)</th>
<th>sIgE to Ara h 1 (kU/L)</th>
<th>Inhibition of dun pea by peanut (%)</th>
<th>sIgE to Ara h 1 (kU/L)</th>
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<tr>
<td>DM</td>
<td>F</td>
<td>8</td>
<td>dun pea</td>
<td>A*</td>
<td>1 year</td>
<td>11</td>
<td>14.9</td>
<td>0.215</td>
<td>17</td>
<td>0</td>
<td>5</td>
<td>14</td>
<td>0</td>
<td>0</td>
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<td>M</td>
<td>10</td>
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<td>A</td>
<td>1 year (positive OC to 0.125 g)</td>
<td>9</td>
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<td>nd</td>
<td>7.5</td>
<td>8</td>
<td>1.5</td>
<td>7.5</td>
<td>0</td>
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<tr>
<td>BM</td>
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<td>5</td>
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<td>Localized U*</td>
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<td>20</td>
<td>&gt; 100</td>
<td>nd</td>
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<td>1</td>
<td>5</td>
<td>1</td>
<td>2</td>
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<td>1</td>
<td>nd</td>
<td>0.8</td>
<td>18</td>
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<td>4</td>
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<td>41</td>
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<td>AO, W, V</td>
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<td>17</td>
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<td>12</td>
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<td>lentil</td>
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<td>&gt; 100</td>
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<td>&gt; 100</td>
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<td>lupine</td>
<td>E, AbP</td>
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<td>nd</td>
<td>9</td>
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**PT to dun pea**

Overall, 23 patients were sensitized to legumes (groups 1, 2b and 2c). Although dun pea and green pea were both variants of Pisum sativum sativum, PTs to dun pea and green pea did not yield the same results. Hence, PT to dun pea was positive in 19/23 cases, while PT to green pea was positive in only 11/23 cases. The specificity of PT to dun pea could be ascertained since it was always negative when there was no sensitization to other legumes (group 2a) (table 3). Moreover, sensitivity was
pea isolate contains more proteins (90%) than green pea (6%). However, there was no correlation between both PTs ($r^2 = 0.060$ and $p = 0.313$), suggesting different allergenic profiles.

**Dun pea-sIgE**

sIgE to dun pea were detected in 18/36 patients (50%), with levels ranging from 0.5-83 kU/L (median 4.9 kU/L). The concordance of PT and sIgE was analyzed in 28 cases. Double positivity was observed in 17 cases and double negativity in 8 cases (total concordance in 89%). Specific IgE to dun pea were detected in one case with negative PT (subgroup 2a, patient VE) and were not detected in 2 cases with positive PT (group 1, patient BM and subgroup 2b, patient FH).

**Cross-reactivity between peanut and dun pea**

To determine whether there was any cross-reactivity between dun pea and peanut, ELISA inhibition was performed. When patients were allergic to peanut, sensitized or allergic to legumes, an inhibition was observed in 9/13 cases (34%-83% inhibition) (table 3 and 4). These patients had sIgE to Ara h 1 in 22/23 cases (96%). The same sIgE were detected in only 6/13 cases (46%) in subgroup 2a with isolated peanut allergy. In group 1 with isolated legume allergy, sIgE to Ara h 1 were detected in 5/6 patients (table 2).

Specific IgE to Ara h 1 were evaluated in peanut-allergic patients according to their lack of sensitization to legumes (subgroup 2a) or the presence of sensitization or allergy to legumes (subgroup 2b and 2c). No sIgE to Ara h 1 were detected in the first subgroup in six out of 13 patients. Specific IgE to Ara h 1 were detected in all patients (17/17) with sensitization or allergy to legumes ($p = 0.003$).

**Allergenicity in different dun pea extracts**

SDS-PAGE separation followed by protein staining of the different pea extracts (green pea, dun pea seed, dun pea flour and dun pea isolate) revealed complex electrophoretic patterns, including components ranging from around 100 to 9 kDa (figure 1A). Some proteins, 70 kDa, 50 kDa, 38 kDa, 28 kDa, 21 kDa, 17 kDa, 14 kDa and 9 kDa, were present in the four extracts. Interestingly, the profile of dun pea isolate was closer to green pea than dun pea seed. Although the electrophoretic profiles of dun pea seed and green pea were different (figure 1A), immunoblot of patient HE with both extracts (figure 1B) revealed a similar allergenic profile, showing that dun pea seed and green pea share common allergens.

An immunoblot with dun pea seed, flour and isolate was performed with serum from patient HE (figure 2A). Proteins of 28 kDa, 17 kDa and 14 kDa were strongly recognized by the IgE in the three extracts. Proteins of 70 kDa, 50 kDa and 38 kDa were strongly recognized in the seed and the isolate, but weakly recognized in the flour. Although present in all three extracts (figure 1A), the 9 kDa proteins were recognized only in the seed extract.

Cross-inhibitions were performed between seed and flour extracts (figure 2B). They showed that 50 kDa and 28 kDa proteins of seed were better inhibited by dun pea flour extract, than by dun pea seed extract. Moreover, all proteins recognized by the IgE in flour extract were inhibited by seed and flour extracts. Finally, the 9kDa proteins more present in seed extract than in flour extract (figure 1A) were still recognized by IgE in the presence of inhibitor flour extract (figure 2B). Taken together, these observations suggest that the epitopes were presented differently in both preparations.

**Figure 1 - A.** Protein staining of green pea (GS), dun pea (DS), dun pea flour (DF) and dun pea isolate (DI) separated by SDS-PAGE. B. Immunoblot of patient HE with dun pea (DS) and green pea (GS). M: marker of molecular weight.

**Figure 2 - A.** Immunoblot of patient HE with dun pea (DS), dun pea flour (DF) and dun pea isolate (DI). B. Immunoblot of patient HE inhibited with dun pea (B1) or inhibited with dun pea flour (B2).
Discussion

Legumes have abundant storage proteins, including the superfamilies of cupins and prolamines (11), explaining the frequent in vitro cross-reactivity (12-14).

Like green pea, dun pea is a variety of Pisum sativum sativum. Allergens of green pea are Pis s 1 (50 kDa vicilin), Pis s 2 (64 kDa convicilin sharing a 65% homology with the former), Pis s 5 (profilin), Pis s 6 (17 kDa PR10-protein), Pis s albumin (26 kDa) and an agglutinin (30 kDa). Pis s 1 and Pis s 2 are major allergens (15). Other possible allergens are 2S albumins, sharing homologies between lentil and green pea (16).

Pis s 1 shares homology with Len c 1 (90%) from lentil, Lup a 1 (52%) from lupine, Gly m 5 (52%) from soybean, Ara h 1 (50%) from peanut. For Pis s 2, the homologies are respectively 68%, 44%, 45%, 45% (17). Besides, Ara h 1 shares homology with Lup a 1 (60% homology), Len c 1 (58% homology), Gly m 5 (57% homology).

Despite the fact that protein profiles are different between dun pea and green pea, the allergenic profiles are very similar (figures 1A and 1B). We can thus postulate that dun pea contains an allergen homolog to Pis s 1. Even if there is only 50% homology between Pis s 1 and Ara h 1, attention is drawn to the 96% of positive ELISA to Ara h 1 in our series of patients sensitized or allergic to legumes. This incidence is much higher than that observed in large peanut-allergic populations (9,18). Furthermore, in patients allergic to peanut, sIgE to Ara h 1 was detected in all patients (17/17) if they were sensitized or allergic to legumes, whilst they were detected only in 7/13 patients who were not sensitized to legumes. These data, together with the results from inhibition of sIgE to dun pea by peanut suggest a higher risk of sensitization or clinical reactions to legumes and to dun pea, in a subgroup of peanut-allergic patients sensitized to Ara h 1.

However, up to now the clinical risk of cross-reacting foods, namely legumes, depends on complex factors (19) and cannot be evaluated by this small series. According to Sicherer, established allergy to more than one legume could indicate higher risk for multiple allergies (19). Out of our 36 patients, eight fulfilled this condition (group 1 and subgroup 2c).

Little information is available concerning the influence of food technologies on legume allergenicity (20). Moreover, detailed information about the technological processes for industrial foods is rarely obtained from the food industries. Flour is obtained by physical processing: dehulling, micro-crushing and extrusion. We have no additional data for the isolate. Immunoblots of serum from patient HE with the same amount of protein amount for each extract illustrate the difference of IgE reactivity with seed, flour and isolate extracts. Hence, (i) allergenic profiles were different between flour and isolate (figure 2A), (ii) IgE recognized the 9 kDa proteins in dun pea seed but not in flour and isolate (figure 2A), (iii) the absence of inhibition of the 9 kDa proteins of seed by flour (figure 2B) confirmed that the 9 kDa proteins present in flour and isolate were no longer able to bind the sIgE, and finally (iv) seed and flour differentially inhibited IgE binding to 28 kDa and 50 kDa proteins in seed (figure 2B). These observations raise the hypothesis that manufacturing processes may be different for the two types of ingredients, thus modifying the allergenicity of native proteins.

Legumes are staple foods worldwide and attention is drawn to the prevalence of legume allergy. Owing to their nutritional properties, they are used increasingly as protein ingredients, and the recent introduction of dun pea flour or dun pea isolate by food ingredient producers has been considered a safe alternative to the use of soybean or lupine proteins. However, the quantity of dun pea proteins included in a 20% enriched steak mince is 17 g, instead of 12 g ingested in a routine portion of green peas. Misleading allegations claim that dun pea products are not allergenic. Since their presence on the labelling may be only notified as “vegetable proteins”, consumers, health services and regulatory authorities cannot currently identify the allergenic risk of dun pea, and widely to all peas. This study documents the in vitro cross-reactivity of dun pea with other legumes and peanut, and highlights some cases of clinical reactions to dun pea in patients allergic to legumes (1) or peanut (3). Further studies should clarify the extent of the risk of pea used as an ingredient.

Acknowledgements

We thank D. Maurice for ELISA and Immunoblots, B. Sansas for his statistical advice and S. Tscheiller, analyst of the Allergy-Vigilance Network, for her technical assistance.

Conflict of interest

CR and SJ are employed by Genclis SAS.

References

Cross-reactivity of a new food ingredient, dun pea, with legumes, and risk of anaphylaxis in legume allergic children


Anomalous cutaneous absorption of allergens as cause of skin prick testing adverse reactions in adult patients. Clinical and experimental evidence

A. Antico¹, M. Arisi², G. Lima³

Introduction

Skin prick test is currently the technique more widely used to diagnose allergic sensitization to common allergens. The fast and painless execution and the high number of allergens tested in the same session are some of the unquestionable advantages of the method. Considering the smallest amounts of allergen injected into the skin (1), the prick test must be considered on the whole a safe diagnostic procedure. If not altogether absent, the risk of systemic adverse reaction is very low.

Some large clinical-epidemiological studies have suggested that the overall risk of inducing anaphylactic reactions by skin prick testing with common allergens is about 0.02% (2,3). The progress on extracts standardization and diagnostic methods has further reduced the rate of reactions with commercial extracts to less than 0.002% (4), being latex or fresh foods more likely to cause adverse events (5-8). Paediatric age, active eczema and high number of allergens tested in poly-sensitized patients have been pinpointed as possible risk factors (9,10).

Systemic reactions are usually mild to moderate in severity and can be easily controlled by recommended therapy (11). No fatalities have been reported in the last decades. Unusual conditions of hyperactivity, an overload of allergens by non-standardized or much more concentrated extracts, or a lot of positive reactions can be seen as a possible explication of some cases of systemic reactions.

Summary

Background. Paediatric age, active eczema and high number of allergens tested in poly-sensitized patients have been pinpointed as possible risk factors of systemic reactions by skin prick testing. As far as atopic eczema concerns, the higher penetration of the allergens into the skin because of the scraping or micro-injuries is an intuitive rationalization. Purpose of the present study is to provide documentary evidence that adverse reactions elicited by anomalous absorption of allergens can occur also in adult patients with apparently normal skin. Methods. Report of some exemplifying clinical and experimental observations. Measuring the inoculum volume into impaired skin and its variability in relation to the variation of the chemical-physical characteristic of the solutions used for the tests by means of a method of direct assay based on the use of a gamma-camera. Results. Localized impairments of the skin permeability can cause a significant increase in inoculum volume by prick-test. Critical amounts of allergens can be introduced into the skin because of the possibility of direct absorption, also without pricking, of allergy diagnostic solutions. The greater water content of the solutions used for prick-testing can significantly increase the inoculum volume. Conclusions. This study adds clinical and experimental evidences that localized impairments of permeability can occur in adult patients with apparently normal skin. Special precautions should be taken when a change of the drops' normal shape and cohesion is seen, because allergy prick-testing in such areas is potentially associated with increased risk of large local or systemic reactions.
As far as eczema concerns, the higher penetration of the allergens into the skin because of the scraping or micro-injuries is an intuitive rationalization. However, a clear demonstration of this probable mechanism does not exist.

Aim of the present work is to report clinical and experimental evidences that an anomalous absorption of a critical amount of allergens, potential cause of systemic reaction, can occur also in adult patients with apparently normal skin. What’s more, we studied the effect on inoculum volume of the variation of the chemical-physical characteristic of the solutions used for the prick-test.

Methods

We report some explanatory clinical observations taken out from our files to prove with documentary evidence that, in some adult patients with impairments of skin permeability, there is the possibility of significant increase of allergens load by absorption and penetration through the skin of diagnostic solutions also without doing prick/puncture tests.

The amount of allergen extract which could penetrate into the skin by a prick test altered by simultaneous absorption of the solution used for testing, has been assessed by means of a method of direct assay based on the use of a gamma-camera. In short, a 50% glycerol-saline solution routinely used as diluent in allergy work was labelled with 99m Tc-pertechnetate (Tc99m). The inoculum volume was calculated with precision measuring the activity of the solution penetrated into the skin by means of a gamma-camera. This assay method and its overall reliability in terms of sensitivity, precision and accuracy, and the results of the assay of the inoculum volume by prick testing have been extensively reported elsewhere in literature (1,12).

The possible variations of the size of inoculum volume in relation to the variations of the chemical-physical characteristic of the solution used for the tests have been studied with the same technique. For this aim, some series of prick test were carried out in 15 health subjects (average age 43 ± 8; 13 female) by means of two glycerol-saline solutions respectively at the concentration of 10 and 50%.

Four rows of prick test were carried out on the volar side of the forearms of each subject (i.e. two rows of 4 prick test for each forearm, the one with 50% and the other with 10% solution, alternating the radial and ulnar side) for a total of 16 tests per person. The data series have been compared by Wilcoxon matched-pairs test.

All patients gave their written informed consent and the study with radioisotopes was then approved by Local Ethical Committee (Del. N. 665, 16.04.96).

Results

Case report 1

This clinical observation concerns a 23-year-old female patient, who had referred to our service because of the onset, for about two years, of perennial rhino-conjunctivitis and asthma. She reported a personal history of atopic dermatitis recovered at school-age and a vague story of food allergy. At the same time, together with the respiratory symptoms, frequent occurrence of widespread pruritus and of recurrent, fleeting episodes of dermatitis of flexural surfaces of the joints, mainly in winter, were started. No active skin lesions were present at the time.

Performing skin tests we noted a fast spread out of the allergen drops put down, and their near complete disappearance, adsorbed by the skin. Actually, the forearm skin was very dry, lackluster with a fine scaling by gentle rubbing and accentuated skin markings in the areas of elbow and wrist folders. Skin test procedure was stopped. To verify the effective penetration into the skin, a drop of the positive control (histamine 10 mg/mL in 50% glycerol-saline solution) was put down on the wrist without pricking. The drop spread out and was adsorbed by the skin, with provocation of a large flare and a number of wheals of different size (figure 1).

Case report 2

Similar case concerning a 32-year old bricklayer with perennial rhinitis and mild asthma. The patient had never suffered from atopic dermatitis or other cutaneous diseases and skin appeared to be normal. Prick-tests were normally carried out. However, soon after skin pricking a slow spreading and adsorption of some allergen drops near to elbow, including house-dust mites extract, was noted. Drops were immediately wiped by blotting paper. Nevertheless a strong reaction, with a very large flare and a lot of wheals of different size involving skin areas of other
Since an IgE-mediated reaction was really improbable, prick tests were quickly performed and the test solution summarily dried by blotting paper. No immediate-type positive cutaneous responses were seen. At the end, as usual, the skin was wiped with a cotton wad wetted of disinfectant solution.

The next morning the patient came to our service because of the occurrence of delayed skin reaction which involved not only the points where the prick-tests were performed, but the entire area of contact where the liquid had been spread and dripped, and clearly absorbed by the skin (figure 3).

**Figure 3 -** Delayed skin reactions to prick-tests with ampicillin and amoxicillin (see text). The shape and the extensive size of the patches reflect the skin areas where the solution drops were put down, spread out and formed rivulets, and where the antibiotics had been absorbed.

**Case report 3**

A 43-year old woman, housewife, addressed to our allergy unit for an episode occurred about two months before, of a severe, delayed generalized skin reaction, presumably a maculopapular rash, resulted from the intake of some capsules of amoxicillin. She was not suffering from atopic diseases, but reported intolerance to the costume jewellery and an episode of mild hand eczema in the past.

The patient was skin tested according to ENDA/EAACI guidelines (13). In particular, skin prick tests with ampicillin and amoxicillin were performed at the concentrations of 0.2, 2, 10 and 20 mg/mL.

Also, in this case we noted that the drops put down did not maintain their spherical shape but spread and formed rivulets on the forearm surface. The skin looked apparently normal.

**Figure 2 -** Spreading and absorption of an allergen soon after skin pricking. A very large flare and a number of wheals of different size involving skin areas of several other prick tests make a reliable interpretation of results impossible.

**Case report 4**

A 19-year-old boy had been sent to our service for persistent rhinitis on progressive worsening with secondary asthma. The respiratory symptoms started at age of 12 about and during the babyhood he suffered from a mild, short lasting form of atopic dermatitis. Also in this case we noted that the drop of extract went slowly losing its spherical form, spread and partially penetrated into the skin. Skin testing was stopped. The forearm skin was dry but no other alterations were seen.

Two drops of the histamine control were put down. Skin prick test was carried out through one of them. Both gave a positive skin response, but the one pricked provoked a strong reaction with a flare of unusual breadth (figure 4).
Adverse reactions by skin prick testing

**Figure 4** - Positive cutaneous responses to two drops of histamine control. The one above is the result of percutaneous absorption without pricking. The one below is the response to a prick-test, which provoked a strong reaction with a flare of unusual breadth.

**Case report 5**

To assess the size of inoculum volume, series of prick tests had been performed on the forearm volar aspects of a number of healthy volunteers with a 50% glycerol-saline solution, routinely used as diluent in allergy work, labelled with Tc99m (1,12). As for the clinical cases previously reported, in one subject out of them (a healthy 64-year-old woman, with apparently normal forearm skin), we observed the spreading and the partial adsorption of one drop of the solution. In this way we had the opportunity to calculate the size of the volume which could penetrate into the skin in an example of prick test modified by the simultaneous adsorption of the solution into the skin area surrounding the pricked point, and to match it with the average volume of inoculum of the prick test carried out on normal skin areas of the same subject.

In this example, the volume of solution penetrated into the skin (0.232 μL) was about 19 folds greater than the average volume of inoculum (0.012 μL) by prick testing in healthy skin (**figure 5**).

**Variability by solutions’ water content**

The use of a solution at higher water content (glycerol-saline solution 10%) produced a significant increase in the size of inoculum volume as compared to one with lower water content (glycerol-saline solution 50% routinely used as diluent in allergy work) in more than seventy percent of the cases (11/15 = 73%). For the remaining cases no significant variations have been observed (**table 1**). The data show great differences between the different subjects. The average increase of the inoculum size has been of about two hundred percent (median = 184), but within a wide range of variability, for one case over seven hundred percent (range 85-765%).

**Table 1** - Average inoculum volume (μL) by prick test carried out with 50% and 10% glycerol saline solution in 15 healthy subjects. In 11 of them, the inoculum size results significantly greater with solution at higher water content (10% solution) compared to more concentrated one (50% solution).

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**Figure 5** - Monitor image of a series of prick tests performed on the forearm with glycerol-saline solution labelled with Tc99m and acquired by gamma-camera. The dimensions of one inoculum, compared to other ones, appear very outsized. The enlarged image shows the spread of solution and at least three different sources of penetration into the skin.

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Discussion

We hypothesize that an impairment, more or less localized and maybe transient of skin barrier-function could provide a reasonable and exhaustive explication of observed phenomena. The most obvious function of the skin is to protect the body against the environmental noxae. The epidermal permeability barrier, which controls the transcutaneous movement of water and electrolytes, is probably the most important protective function of the skin. Most part of this barrier function resides in the stratum corneum, composed by many layers of anucleate corneocytes embedded in an intercellular lipid matrix. A second level of defense is formed by the tight junctions of the keratinocytes, and by the lamellar bodies of the stratum granulosum resulting in the formation of an impermeable, lipid-containing membrane. The permeability barrier is largely represented by the epidermis. When the epidermis is disrupted, the underlying dermis is almost completely permeable. It is important to remark that even minimal injuries predispose to more penetration of fluids or other materials applied topically on the skin surface (14).

The surface of the skin is sheltered by a lipid film, composed of both sebum and the lipids of the epidermal cells (15). This film acts as a hydrophobic, low wettability surface. For this reason a fluid put on the skin will tend to minimize contact with the surface and will form a compact liquid droplet. On healthy skin water drops maintain their spherical shape, will not roll off and not fall even if the forearm is tilted. Because in normal conditions (at least for not lengthened applications), there is not significant absorption of the aqueous liquid or other substances put on the skin, pricking through the drop is necessary to produce a micro-lesion by which a tiny amount of solution is introduced into the skin.

The homeostasis of the epidermal permeability barrier is finely and actively regulated. Impairment or loss of barrier-function are primary pathophysiologic factors in a number of skin diseases, including atopic dermatitis, ichthyosis and many other xerotic skin conditions (14). Abnormalities in lipid processing metabolism and genomic defects concur to the skin barrier abnormalities in atopic dermatitis (14,16). Filaggrin gene mutations and ineffective keratinocyte differentiation, decreased levels of ceramides and pyrrolidone carboxylic acid result in abnormal keratinization of skin, abnormal lipid organization and deficiency of the natural moisturizing factors. Alterations in sebum secretion and chemical composition of skin surface lipid are a common feature in atopic dermatitis and several inflammatory chronic skin diseases (15). Because of these structural and functional changes, permeability barrier function is impaired displaying both increased trans-epidermal water loss and lowered water-binding capacity. Atopic skin proves very dry and more vulnerable to the penetration of exogenous substances.

As a consequence of alterations and reduction of its lipid film, in atopic dermatitis and other xerotic skin condition, the normal hydrophobicity of the skin surface is frequently lost. In the case, water and fluid drops put down on the skin cannot maintain their form, but spread out. As in clinical cases reported, this occurrence should be considered a warning of significant damage of the skin with impaired permeability barrier function, allowing for a fast substances penetration (15).

Occasionally, spreading and dripping of extract drops put on the skin can be seen also in some patients with no structural change or impaired function of epidermis. Soaps, synthetic detergent or bath foam, but also some cleansing and moisturizing cream used for cleanliness and body care can deplete or damage the lipid film. In this cases, water drops can spread out and form rivulet. If the damage is only limited to the superficial external lipid film, there is no significant water adsorption. That is because skin barrier-barrier function is mainly (although not exclusively) fulfilled by underlying corneus stratus (the so-called “brick and mortar” structure), and the damage of the corneus stratum is a necessary condition for the impairment or loss of skin barrier-function. Barrier creams (topical formulations used to place a physical barrier between the skin and external noxae) could provide a protective film, replacing the function of the natural outside hydrolipidic film which covers the skin. However, prick-puncture tests produce a micro-lesion by which the liquid is introduced into the skin diffusing through the epidermis. For this reason, just restoring the function of the external lipid film is not enough to prevent an abnormal penetration and spreading of the allergen solutions.

However, housekeeping products, soap with high content of free alkali and/or harsh chemicals in cosmetics can go deep into the skin dissolving the lipids of underlying epidermal layers, impairing skin barrier and increasing permeability (17). This is probably the case of the housewife we reported (case 3). Here we must stress the point that, if the patient had had an IgE-mediated sensitization, in all probability, skin prick testing would challenge a severe anaphylactic reaction.

The normal skin acts as a two-way barrier to prevent the inward or outward passage of water and electrolytes. Studies on drugs delivery by transdermal patch demonstrate that the penetration of substances through the skin surface depends upon different factors, which include skin conditions (e.g. injured or abraded skin surfaces, hydration, etc.), age, physical and chemical characteristics of considered substances and time of application. The absorption through the skin acts by a slow process of passive diffusion through the corneum layer. Defects in epidermal permeability barrier, by skin diseases or injuries enhance and accelerate the diffusion processes (18).

Clinical cases reported demonstrate that the absorption of the glycerol-saline solution normally used for skin prick tests can be
really fast. Moreover, puncture-prick tests carried out on dry, injured skin seems to enhance considerably the fluid penetration and diffusion. As a matter of fact, the prick-test in the reported cases provoked skin reactions of unusual breadth (cases 2 and 4). In a similar situation (case 5), we have demonstrated that the volume of solution penetrated into the skin was far higher than the mean size of inoculum in normal skin. The monitor image visually explains the spread of the solution into the skin and the scale of the phenomenon (figure 5).

Water is an effective penetration enhancer. Results of our study show that an aqueous solution produced a significant increase in the size of inoculum volume, as compared to one with low water content. In clinical practice, it means that when prick tests were carried out using extemporary, aqueous extracts or food with high water content (like milk or some fruits), also on healthy skin an amount of allergens much higher than expected can be introduced into the skin. In conditions of pathologic skin with altered permeability, critical amount of allergens, sufficient to induce systemic reactions in a sensitized patient, could be reached with a single prick test.

In conclusion, we have added clinical and experimental evidence that prick-testing in patients with atopic dermatitis and other skin diseases or conditions with impaired permeability of the skin is a risk procedure. Areas of normal skin should be carefully chosen to prevent large, scattered local reactions for which test results could be very difficult to interpret, and suitable precautions should be taken to avoid risk of systemic allergic reactions.

References

Can esophageal dilation be avoided in the treatment of severe esophageal stricture caused by eosinophilic esophagitis?

D. Silva¹,², F. Santos³, S. Piedade¹, M. Morais-Almeida¹

Introduction

In the last decade, eosinophilic esophagitis (EoE) has been increasingly recognized in clinical practice (1,2). This is an inflammatory immune-mediated condition, with an eosinophilic-predominant inflammatory infiltration, characterized by esophageal dysfunction symptoms (2,3). EoE is also considered an antigen driven disease (4). Food allergens play an important role in pathogenesis of the disease, but aeroallergens have also been implicated as co-factors contributing to the development of EoE (5). Since 2007, two consensuses have been performed concerning its diagnosis and treatment (2,6), and recently an evidenced based approach was used to assess the strength of these recommendations (3). This chronic disease, presenting with persistent and relapsing symptoms (2), differs in clinical presentation accordingly to age. Diagnosis is challenging particularly in children, which can lead to a diagnostic delay that reached up to 6 years in some cohorts (7). In a recently published large retrospective, cohort of patients with eosinophilic esophagitis, the likelihood of a fibrostenotic disease, defined by the presence of esophageal rings, narrowing or strictures, doubled for every 10 years in age increase (8). This is probably dependent on the persistent inflammatory nature of the disease followed by the appearance of fibrosis, if no anti-inflammatory measures are initiated (7). The main treatment of EoE are corticosteroid, as well as dietary intervention indicated in some pa-

Summary

Eosinophilic esophagitis (EoE) is an inflammatory immune-mediated disease with predominant eosinophilic inflammation characterized by the presence of esophageal dysfunction symptoms. Treatment delay can be associated with disease complications, like esophageal strictures, that can justify the use of invasive procedures which are not deprived of side effects. We present a case report of a 14 year old child with severe esophageal stricture secondary to EoE, that was treated with topical and systemic corticosteroid before any invasive procedure was considered. After 26 weeks of medical treatment, significant improvement of esophageal dysfunction occurred with histological remission and stricture resolution. In patients with severe esophageal strictures secondary to EoE, the need for esophageal dilation procedures should be considered only after anti-inflammatory treatment.
Can esophageal dilation be avoided in the treatment of severe esophageal stricture caused by eosinophilic esophagitis?

Blood eosinophilia (1210/mm³), elevated serum total IgE (sIgE) including milk, egg, soy, cereals, fish, seafood, nuts and seeds (UniCAP®, Thermo Fisher Scientific, Uppsala, Sweden). The patient showed sensitization to sesame and sunflower seed through skin testing and specific IgE (sesame 2.07 kU/L; sunflower 1.10 kU/L). Specific IgE were positive to milk (1.35 kU/L) and milk proteins (α-lactalbumin 1.23 kU/L; β-lactoglobulin 1.23 kU/L; casein 0.55 kU/L), cereals (wheat 2.36 kU/L; corn 0.50kU/L; rye 1.86kU/L; barley 2.49 kU/L and oatmeal 2.03 kU/L), nuts (almond 0.57 kU/L; walnut 0.53 kU/L) peanut 1.85 kU/L and mollusks (squid 1.54 kU/L; snail 1.11 kU/L). The patient started treatment with a course of oral corticosteroids (prednisolone) during 1 month, associated with topical fluticasone (1000 µg/day), montelukast (10 mg/day). Esomeprazol (40 mg/day) previously started after the first endoscopy was kept. An allergy testing-directed elimination diet to sesame and sunflower seeds was also recommended. Six weeks after treatment, reassessment showed clinical improvement. Endoscopy, performed after a 2-month course of proton pump inhibitors treatment, still maintained circular ring pattern with few longitudinal furrowing and white exudates plaques but the stricture was now traversed by the ultra-slim videogastroscope (figure 1.b). Biopsies were performed on the upper, medial and distal esophagus, stomach and duodenum (2 fragments obtained from each location). Histopathology showed, in all the esophageal segments, infiltration by eosinophils (> 25 eos/HPF) and microabscesses. Treatment with topical corticosteroid, montelukast and proton pump inhibitor was kept for more 20 weeks. Eviction diet continued to be recommended, but was not strictly followed by the patient. After 26 weeks of treatment, food impaction or dysphagia symptoms ceased and in the upper digestive endoscopy no esophageal narrowing or stricture was observed, esophageal mucosa was normal and an 8.8 mm diameter videogastroscope progressed easily (figure 1.c). Histopathology analysis of the esophagus found rare eosinophils in the mucosa, both in the proximal and distal thirds. Topical corticosteroid was maintained and clinical and histological remission was kept after one year of clinical and endoscopic follow-up.
Discussion

In this case report a severe esophageal stricture due to eosinophilic esophagitis was completely resolved in 26 weeks with only medical treatment, avoiding mechanical dilation procedures and their inherent risks.

The prominent esophageal eosinophilia that characterizes EoE leads to tissue remodeling, namely subepithelial fibrosis and fibrovascular changes, which predisposes to the formation of esophageal rings and strictures and increases tissue frailty (13). Esophageal dilation allows a mechanical immediate relief of symptoms but has no effect on the underlying esophageal eosinophilic inflammation, therefore stricture recurrence can occur (2,10,12). Moreover this procedure is not deprived of risks (13-15). When accessing the rate of complications of a series of 293 dilation sessions, 9% had deep mucosal tears and 1% risk of perforation (16). Furthermore, in a cohort study, 74% of the questioned patients reported retrosternal pain after the procedure (12). A proximal location in the esophagus and dilation of small-diameter strictures were reported to be associated with higher risk of complications (16). Additionally, when esophageal dilation was used in an initial therapeutic approach in a cost analysis model it was found to be more costly than topical corticosteroids (17). The use of steroid therapy as first-line treatment before esophageal dilation can be an option, though no consensus exists regarding how long medical therapy should be performed before resorting to esophageal dilation and there is lack of evidence ascertaining which esophageal strictures will reverse with only pharmacological and/or dietary therapy.
(2,3,13). In a fourteen year follow-up study of thirteen adults with steroid-naive eosinophilic esophagitis treated only with endoscopic dilation and proton pump inhibitor therapy, at least 3.2 dilations were performed during the first year and dilations maintained at every two years (18). By other side, in the Swiss EoE cohort study with 5 year follow-up period, the use of swallowed topical steroids was associated with lower risk for long-term bolus impactions (OR 0.411, 95%-CI 0.203-0.835, p = 0.014) (19). We present an observational evidence of the isolated use of pharmacological treatment in the improvement of esophageal dysfunction, histological remission and severe esophageal stricture resolution. The clinical favorable evolution without the need of an invasive procedure raises the question if, in this predominantly inflammatory driven disease, the need for esophageal dilation procedures could be avoided or delayed. Anti-inflammatory measures, like systemic and topical corticosteroids use, isolated or combined, and proton pump inhibitors can significantly influence the prognosis of the disease, even in its more severe forms (3). Dietary interventions, in a recent systematic review (9), have been shown to be effective in inducing histologic remission, specially with elemental and six-food elimination diets. These results were not consistent for allergy testing-based food elimination (9). Patient adherence to dietary restrictions can be difficult, particularly in older children’s and adults (20), as occurred in this case report. Therefore, the use of more specific elimination diets, like four-food elimination diet, that includes the most common food triggers, and allergy testing-based food elimination could be useful for long term adherence and disease control (9,21). Allergy testing, using skin prick test and specific IgE, still have a limited role in detecting a particular antigen precipitating EoE (22). New tools namely complement resolved diagnosis (CRD) could be useful to assess aeroallergen and food sensitization. Recently, two studies (23,24) assessed, in EoE patients cohorts, sensitization to cross-reactive allergens using CRD. They observed two main different cross-reactive patterns of sensitization, one to PR-10 (24) and the other to profilin (23). Indeed, both suggested that pollen and food sensitizations may contribute to esophageal inflammation in EoE patients. Therefore, CRD could provide more insight into sensitization patterns, identify additional food allergen sensitizations and help in targeting allergy-testing directed elimination diet. However, the clinical utility and efficacy of CRD in guiding specific elimination diet is still not settled (24).

This case report enlightens that, in patients with eosinophilic esophagitis complicated with severe strictures, more evidence is needed to fully understand the role of topical corticosteroid treatment and dietary therapy in comparison with esophageal dilation procedures in the clinical and histological remission of the disease.

**Conclusion**

In patients with esophageal strictures secondary to eosinophilic esophagitis the need for esophageal dilation procedures could be avoided, even in severe strictures, if systemic and topical anti-inflammatory treatment is first implemented. Prospective studies are needed to compare these interventions, considering patient-reported outcomes, complications and long-term follow up to monitor disease relapses.

**References**


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