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Allergic rhinitis and associated pathologies: the rationale for steroid options

A murine model of cow's milk protein-induced allergic reaction: use for safety assessment of hidden milk allergens

The impact of air pollution on hospital admission for respirarory and cardiovascular diseases in an oil and gas-rich country

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Allergic rhinitis and associated pathologies: the rationale for steroid options

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Key words

Upper airway inflammation, atopy and sleep disorder, nasal polyps, upper viral infection

Summary

The aim of this review article is to provide greater insight into the relationship between allergic rhinitis and the three most frequently diagnosed conditions of exacerbating viral infections, chronic rhinosinusitis with polyps and obstructive sleep apnoea syndrome. The alleged physiopathological effects of steroids are also investigated within the scope of this paper. Regarding the exacerbating viral infections, seems to establish a dynamic and counter relationship between the load and nature of the viral infection on one hand and widespread and pre-existing allergic inflammation on the other. If chronic rhinosinusitis with polyps and allergic rhinitis present overlapping picture of inflammatory cell and cytokine, the etyiological relationship between the two conditions appears to be influenced by the type of antigenic stimulus. Allergic rhinitis can influence the presence of OSAS through both obstructive and inflammatory mechanical factors. Topical corticosteroid therapy is a promising candidate as a new therapeutic tool able to improve symptoms and quality of life in patient with chronic rhinosinusitis with polyps and obstructive sleep apnoea syndrome. Other study are necessary to elucidate relationship between corticosteroids therapy and hypothetical benefit effect on viral infection when concomitant atopy in patient.

Introduction

The ARIA guidelines were the first ever to recognise the association of allergic rhinitis and its risk factors with the onset and severity of bronchial asthma, involving the upper and lower airways as a single entity, though they present distinct organ symptoms and are treated differently (1). Similarly, other conditions (e.g. chronic rhinosinusitis with and without nasal polyps, vocal cord dysfunction, secretory otitis media and viral infections) have been identified as co-morbidities in subjects with allergic rhinitis.

The aim of this paper is to provide greater insight into the relationship between allergic rhinitis and the three most frequently diagnosed conditions of exacerbating viral infections, chronic rhinosinusitis with polyps and obstructive sleep apnoea syndrome. The alleged physiopathological effects of steroids are also investigated within the scope of this paper.

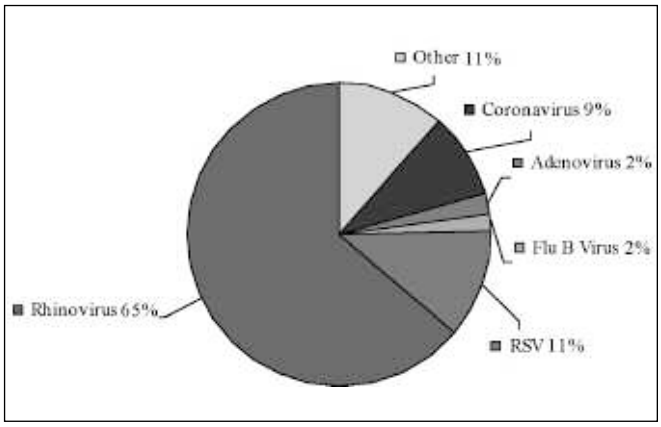
It has long since been established that allergic reactions occur in two separate phases, the first of which is triggered within a

few minutes of exposure to allergen-induced histamine release with arachidonic acid metabolites (leukotrienes, prostaglandins and thromboxanes) progressing into a late stage reaction developing 6-12 hours once exposed to mast cells, T-lymphocytes, basophils and eosinophils.
Activation of these cells produces Th2 cytokines which in turn are responsible for activating endothelials and epithelials, thereby inducing endothelial adhesion molecules like ICAM-1 to be expressed along with vascular cell adhesion molecules, such as VCAM-1. ICAM-1 is a surface glycoprotein which normally directs leukocyte traffic and regulates its accumulation into the inflamed site through the cell-surface ligand of the lymphocyte function-associated antigen (LFA)-1 and macrophage-1 antigen (MAC)-1. Epithelial activation is associated with the production and release of numerous immunoregulatory cytokines which include RANTES, macrophage proteins, inflammatory (MIP)-1·, monocyte chemotactic proteins (MCP)-1, IL-8, and eotaxin (2).
The inflammatory mechanisms described above are activated and detectable in viral infection-mediated exacerbations of the upper airways, in chronic rhinosinusitis with polyps and in nocturnal apnoea syndrome, leading to the hypothesis of a set of mechanisms common to allergic rhinitis and the aforementioned conditions.
The corticosteroid molecules routinely used in the local treatment of allergic rhinitis with good clinical outcomes (1) include beclometasone propionate, flunisolide, budesonide, triamcinolone acetomide, fluticasone propionate, mometasone furoate and more recently fluticasone furoate. Topical corticosteroids provide elevated selectivity for the glucocorticoid receptor and low oral bioavailability (3-5). The effectiveness of fluticasone furoate appears interesting also on ocular symptoms, widening the therapeutic spectrum of this drug class to the naso-lacrimal duct (3,6-8).The mechanism of action of corticosteroids involves changes in DNA molecule which results in a down regulation of transcription, of pro-inflammatory proteins, in an enhanced production of anti-inflammatory proteins, which limits recruitment and action of inflammatory cells, as well as in a reduced secretion of pro-inflammatory mediators during the late phase allergic response (2,9-11).

Allergic rhinitis and viral exacerbations

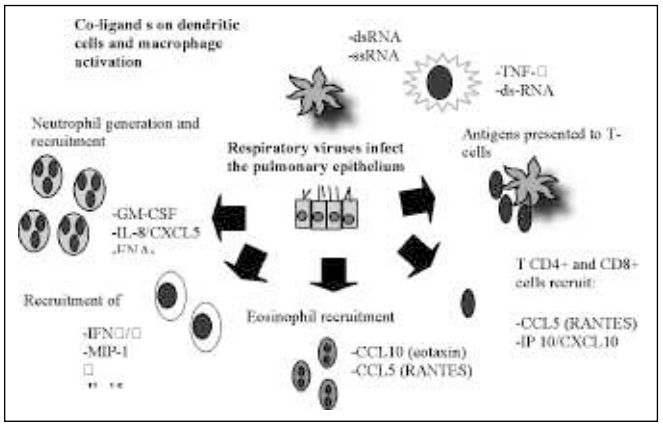
Airway viruses are powerful stimulants of chemokine and cytokine production. These, rather than the cytopathic impact of the virus itself, are the real culprits of the pro-inflammatory effects of respiratory viral infections (12).
In one study on spontaneously virally infected airway mucosa the majority of the documented cases were caused by rhinoviruses followed by coronaviruses, flu B virus, and respiratory syncytial virus (12) (Fig. 1).
For the most part, the data in the literature come from experimental nasal and/or bronchial inoculations. In worsening asthma, rhinovirus was the most frequent causative pathogen with hospitalisations correlating with the seasonal peak of the infection (14).
More than 90% of rhinovirus serotypes (15,16) are utilized the ICAM-1 adhesion molecule that trought cell receptors and ligands enable the virus to penetrate a host cell and insert its DNA into the host's genome eventually leading to infection (17). Rhinovirus infection induces ICAM-1 expression, thereby making epithelia susceptible to further viral spread (18).
Rhinovirus infection typically causes common cold symptoms which include rhinorrhoea, nasal congestion, sneezing, sore throat, coughing and headache.
Experimentally infected samples of bronchial epithelial cells along with slight cellular damage reveal a pattern of immunological responses, showing that rhinovirus acts by causing clinical worsening through mechanisms not associated with cell damage (19).
Studies based on genetic expression performed using infected epithelial cells suggest that viral replication induces cytokine and chemokine production which are required for recruiting inflammatory cells for the antiviral response: IL-1, IL-6, IL-8, GM-CSF, eotaxin and RANTES (19-22). The secretion of these mediators can

Figure 1 - Adapted from Edwards MR et al. New treatment regimes for virus-induced exacerbations of asthma. Pulmonary Pharmacology & Therapeutics 2006; 19: 320–334 (13)



contribute to virus-induced activation mechanisms and inflammatory cell recruitment (Fig. 2).
This not only means that the epithelial cells are a target for a potential virus reservoir, but also the site and source of a preliminary inflammatory response.
The cellular response to rhinovirus which takes place in this way is both innate and adaptive. T-cell recruitment (adaptive response) can contribute to the clearance of the virus through Th1 cytokine production, including IFN-γ e IL-12. Indeed, the production of RANTES and protein inducing IFN-γ (IP-10) promotes chemotaxis of Th1 cells (23). IFN-γ plays a crucial role in the protection of the host by promoting inflammation which acts as a chemotactic factor towards eosinophils (24) and perhaps by increasing basophil and mast cell histamine release (25).
Besides the production of specific IgA, IgM, IgG, in experimental models, the B lymphocyte response to viral inoculation induces a rapid increase in total IgE sera in subjects with allergic rhinitis with no evident increase in specific IgE (26).
Apart from histamine release (27), leukotriene C_4 is another mediator whose levels are elevated in nasal lavage mucus and debris during rhinovirus infection (28).
The influence which atopic status and specific sensitisations have on the airway responses to viral infections is still under investigation.
There are important differences in the immunological mechanisms which are activated in atopic and non-atopic individuals. Atopic individuals show elevated histamine release and reduced levels of IL-10 during the acute phase, and higher inflammatory cytokine levels of IL-1β, IL-6, RANTES and ICAM-1 along with the prolonged eosinophilis of the airways during the convalescence phase (12). The reduced concentration of IL-10, a powerful anti-inflammatory cytokine which inhibits the synthesis of both Th1 and Th2 would enhance a Th2 anti-inflammatory response (29).
According to the premise that the mechanisms which are the basis for the link between viral infection and atopy for rhinitis and allergic asthma are similar (30), it is clear that a typical Th1 anti-viral immune response in atopic individuals can be inhibited by a pre-existing Th2 (31) environment, thereby favouring the persistence of viral inflammation. Indeed, compelling clinical evidence demonstrates the inverse relationship between IFN-γ production and synthesis of Th2 cytokines like IL-4 and IL-5 (30,32).
Additionally, an IgE increase towards airborne allergens has been considered to be a marker of a greater risk factor for the worsening of both the upper and lower airways during an inflammatory response to viral infections (33). On this point, the analysis of experimentally induced rhinovirus infection in adults with mild asthma reveals that a subset of patients whose total IgE is greater than 300 UI/mL present a significant increase in symptoms involving the upper and lower airways compared to healthy controls (33).
One possible explanation of this evidence could lie in the demonstration that IL-13 and other Th2-like cytokines are able to increase ICAM-1 expression on *in vitro* rhinovirus-infected epithelial cell lines (EC line) (34) and that in epithelial cells harvested from nasal brushing in atopic individuals ICAM-1 expression is higher than in healthy subjects; this increase can be ascribed to the exposure to an allergen of an atopic subjects (35).
On one side exposure to an allergen in sensitised subjects can favour viral infections thereby increasing ICAM-1 expression, while as regards the adaptive immune response, the clinical outcome to the respiratory viral infection in allergic subjects depends on how the individual immune response balances the load and type of virus as well as the severity of the pre-existing atopic inflammation. (30) (Tab.1).
Some *in vitro* studies have shown that glucocorticosteroids can block virus-induced pro-inflammatory mechanisms in the airways at an epithelial level by means of corticosteroid-induced down-regulation of ICAM-1 expression by epithelial cells. Indeed, treatment with corticosteroids can inhibit the up-regulation of rhinovirus-induced ICAM-1 (37-40).

Figure 2 - Adapted from Edwards MR et al. New treatment regimes for virus-induced exacerbations of asthma. Pulmonary Pharmacology & Therapeutics 2006; 19: 320–334 (13)



Other data show that glucocorticoids can reduce basic ICAM-1 expression and its subsequent induction on exposure to allergens (40).
Similarly, corticosteroids inhaled *in vivo* are able to reduce ICAM-1 expression in bronchoalveolar lavage cells in mild asthmatics (41-44).
Hence, corticosteroids limit the inflammatory action of rhinovirus, not only by inhibiting ICAM-1, but also by reducing cytokine production (Fig.3).
Corticosteroids indeed interfere with the inflammatory process by binding to specific cytoplasmic receptors which: 1) interfere directly with the nuclear -kB factor (NF-kB) and preventing this molecule from attaching itself to DNA and subsequently leading to the release of pro-inflammatory molecules and 2) repress the transcription gene of pro-inflammatory molecules through glucocorticoid-responsive elements, or induce the activation of anti-inflammatory molecules.
For example, pre-treatment with corticosteroids reduces rhinovirus-induced IL-6 production by bronchial epithelials (46), through suppression of the IL-6 gene promoter sequence or reduces IL-8 production through a trans-repression mechanism, inhibiting activator protein-1 (AP-1) and nuclear-kB factor (NF-kB) translocation inside the nucleus, which is required for IL-8 production (41-44).
Interestingly, however, in virus-induced exacerbations of asthma inhaled corticosteroid therapy produces a poorer clinical response, suggesting that mechanisms of steroid resistance develop during worsening,. This circumstance could be related to the increase in nuclear activation factors like the virus-induced activator protein-1 (AP-1) and nuclear-kB factor (NF-kB) (51).
Papi et al. (45) demonstrated that high corticosteroid concentrations do not affect rhinovirus replication or the ability to infect cell lines, which supports the hypothesis that corticosteroids negatively influence rhinovirus replication.
Likewise, Farr et al (52) investigated the role of prednisone (30 mg twice daily) or intranasal beclometasone (168 mg twice daily) administered 3-4 days prior to the rhinovirus challenge which was repeated on 5 successive days. Treatment successfully reduced congestion, rhinorrheoa and kinin production for up to 48 hours after virus inoculation, though any improvement ceased after stopping steroid therapy, suggesting the drug's lack of carryover effect. The limited amount of experimental data on viral infections of the upper airways and on the clinical effectiveness of topical steroids available to date does not t provide a definitively clear picture of the associated risks and of the efficacy of the treatment (18,53,54).

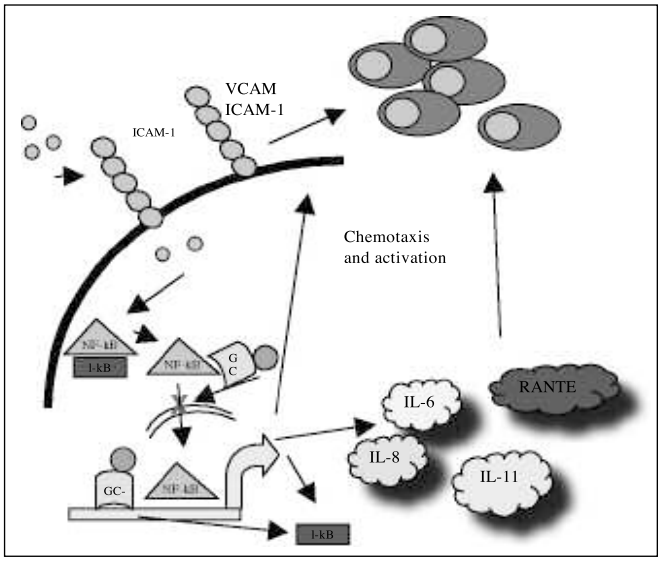
Table 1 - Cellular mechanism of susceptibility to the effects of rhinovirus infection (RV) in asthmatics

	Control	Asthmatics
RV ligand:		
ICAM-1 expression	Low	High
Epithelium integrity	Intact	Altered
Post-RV ligand		
IFN-β response	Early,	Deficit
Cellular lysis VS apoptosis	good	Apoptosis damaged Increase in cellular lysis
Release of inflammatory mediators	Present	Increased (?)
Immune response		
Neutrophil recriutment	Present	Increased
Thl response	Poor (IFN-γ)	Lacking (IFN-γ)

Allergic rhinitis and chronic rhinosinusitis with polyps

In the general population, the prevalence of chronic rhinosinusitis is 15.5% (55), while that of nasal poliposys ranges

Figure 3 - Adapted from Papi A., Nikolaos G. Papadopoulos, Degitz K, Holgate S. T., Johnston S. L. Corticosteroids inhibit rhinovirus -induced intercellular adhesion molecule- 1 up-regulation and promoter activation on respiratory epithelial cells. J Allergy Clin Immunol 2000;105:318-26 (45)



between 1-4%, with a probable multi-factor inflammatory mechanisms which is still being studied (56-62).
Chronic rhinosinusitis with nasal polyps currently presents as a chronic inflammatory disease of the paranasal sinuses, associated with Th2 inflammation, increased numbers of eosinophils (63), presence of mast cells in polyps which are often degranulated (64,65), and local production of polyclonal IgE which often does not correlate with the patient's allergic status (66).
Unlike chronic rhinosinusitus without nasal polyps which in immunological terms presents with a prevalent Th1 profile with elevated levels of interferon-γ (IFN-γ) and transforming growth factor-β (TGF-β), nasal polyps are characterised by eosinophil inflammation (55) with elevated concentrations of eosinophil cationic protein (ECP), with eotaxin cellular activation markers and IL-5 cooperating in eosinophil recruitment and activation. The activated eosinophils infiltrate the nasal polyps producing toxic mediators as well as a variety of chemokines, cytokines and growth factors whose action probably reduces apoptosis and favours increased tissue infiltration through an autocrine mechanism.
It is widely accepted that eosinophils are one of the markers of allergic inflammation.
In patients with nasal polyps and concomitant allergic rhinitis, eosinophils seem mainly attracted by the release of IL-5. In contrast, in the absence of allergy, recruitment appears to correlate with GM-CSF release (67). Nevertheless, the eventual eosinophil influx seems to be identical for both atopic and non-atopic subjects.
Factors associated with chronic rhinosinusitis with polyps are aspirin intolerance (68-73) where 36-96% of sufferers have polyposis, asthma (26-42% of asthmatic with nasal polypolsis) (59), genetically pre-disposed to chronic rhinosinusitis with nasal polyps and environmental factors, especially cigarette smoke (74-77).
When an allergy clinic medical records of almost 5,000 patients were re-examined, the prevalence of nasal polyposis was 4.2% (78), highest among asthmatics (6,7%).

The aetiology of polyposis has been ascribed to to allergy, but this has never been definitely established (79). Between 0.5% and 4.5% of subjects with allergic rhinitis present with nasal polyposis (78,79), a prevalenche that matches that of general population (80). In children, the prevalence of chronic rhinosinusitis with polyps shows a wide variability ranging between 0.1% (78) and 25.6%. (81) of cases.
On the other hand the prevalence of allergic sensitisation in patients with nasal polyposis varies between 10% and 64% (79-84).
If subjects with nasal polyposis and controls with chronic rhinosinusitis are compared for allergic sensitisation a major prevalence of sensitisation to perennial allergens emerges in the former and seasonal allergens in the latter (85).
Though nasal polyposis is classified as showing two different histopathological subtypes, showing a predominance eosinophils and neutrophils, respectively, atopic status did not significantly differ between the 2 subsets, being 62.7% and 81.8% respectively (86).
In contrast with studies reporting that atopy is more frequent in patients with nasal polyposis other studies have not been able to confirm this finding (87-89). In a recent paper, Bachert et al. (56) demonstrated that the presence of atopy, based on allergy testing with airborne allergens, does not correlate with total IgE or IgE antibodies present in nasal polyp tissue and that atopy has no impact on IL-5, IL-4, eotaxin, LTC4/D4/E4, ECP levels nor on the number of eosinophils in nasal polyp tissue. Moreover, Wagenmann et al. (90) demonstrated that Th1 and Th2 cytokines increased esinophils in nasal polyps irrespective of allergic test results. This finding was confirmed by the observation that IL-5 concentration in nasal polyps, which is significantly higher than in controls, correlates independently with atopic status (55). By contrast, Hamilos et al (91) identified different cytokine content in polyp tissues samples collected from allergic and non-allergic subjects.
Although the relationship between allergy and nasal polyps is not clear, from clinical point of wiev the sympton score does not have increase effect.
Indeed, it has been demonstrated in atopic subjects with nasal polyposis that allergen exposure does not correlate with sectioned polyps, clinical scores and the frequency of worsenings (92,93).
The histological findings of a chronic inflammatory infiltrate made up of lymphocytes, plasma cells, eosinophils and respective cytokines suggest a chronic inflammatory type mechanism. Research is underway to isolate the agents responsible for inducing and/or favouring persistent inflammation of paranasal sinuses.
Several micro organisms have been investigated to determine their causal role in chronic rhinosinusitis with polyps. Ponikau et al (94) reported that fungi were present in 96% of 210 patients evaluated for rhinosinusitis. Braun et al. (95) found a comparably high incidence of fungal colonisation in chronic rhinosinusitis sufferers (91%), but together with Ragab et al. they demonstrated that the colonisation occurs frequently in healthy controls (91-100%) (96).

Other pathogens frequently seen in mucus from patients with nasal polyposis are bacteria, especially Staphylococcus aureus (66,97,98).
Van Zele et al. documented that Staphylococcus aureus colonises the centre of middle meatus prevalently in patients with nasal polyposis (64%) compared to those with chronic rhinosinusitis (27%) and healthy controls (33%) (97). Colonisation by Staphylococcus aureus is paralleled by IgE specific for Staphylococcus aureus-derived enterotoxin. The tissue concentration of specific IgE towards Staphylococcical enterotoxinin the nasal polyposis is associated with asthma and aspirin intollerance. Similarly, elevated levels of eosinophils infiltrating nasal polyps and IgE production were detected in this group of patients.
This IgE production appears to be polyclonal, indicating that the S.aureus-derived enterotoxin can behave as a superantigen and activate large subpopulations of T-lymphocytes (56). Besides the enterotoxin, these bacteria express a number of surface proteins like Protein A (SpA) which have the potential to interfere with host defence mechanisms (99,100).
A recent study revealed (101) different temporal and immunological stimulations by various S.aureus-derived products: while staphylococcal enterotoxin is activated through the release of several immunoregolatory and proinflammatory cytokines in the late stage (after approximately 24 hr) favouring a Th2 type pattern, Protein A (SpA) induces the release of mast cell mediators, namely histamines, the leukotrienes LTC4/D4/E4 and prostaglandin PGD2 after only 30 minutes. These data highlight the influence of S.aureus-derived products on nasal polyposis inflammation inducing mast cell degranulation and T-cell activation.
Another extremely interesting chapter in understanding the natural history of chronic allergic rhinitis and rhinosinusitis with and without nasal polyposis is the remodelling in the upper airways (102).
Similarly to bronchial asthma, chronic rhinosinusitis with polyps shows epithelial damage, basement membrane thickening and oedema, at times, with extensive fibrotic tissue (103). A recent study evaluated the presence of MMP-7, MMP-8, MMP-9 and tissue inhibitor of metalloproteinases (TIMP) in chronic rhinosinusitis with polyps, suggesting the role of an imbalance between metalloproteinases and their natural inhibitors (104,105).
By stereologically testing the vascular surface and the density of nasal tissue volume in rhinitic and non-rhinitic subjects, some authors have demonstrated a lack of vascular remodelling in the mucous membrane of allergic subjects (106, 107), whereas others hypothesise increased angiogenesis at a nasal level (108,109).
All authors agree, however, that limited damage to nasal allergic rhinitis mucosa is indicative of extensive remodelling of bronchial mucosa in asthmatics (104).
In the extensive review of the literature on the treatment of nasal polyposis, the European Position Paper on Rhinosinusitis and Nasal Polyposis (55) stresses the importance of disease management recommending steroid therapy as the mainstay of treatment for this condition. Substantial evidence exists supporting the efficacy of topical corticosteroids in reducing the size of nasal polyps (110,111). However, certain patients are either poor responders to steroid therapy or develop resilience to it.
Consistent with studies of the lower airways which demonstrate that glucocorticoid-insensitive asthma is associated with a significantly elevated level of GRβ$^+$inflammatory cells (112-114), the same mechanism has been hypothesised for nasal polyps as well. Hamilos et al. underline an association between GRβ expression and glucocorticoid insensitivity in nasal polyposis and in particular in patients with aspirin intolerance. Specifically, the authors report an inverse correlation between GRβ basal expression and post-corticosteroid therapy reduction of eosinophils, T-lymphocytes and the expression of VCAM-1 and mRNA cells positive to IL-4 (115). However, other authors having failed to determine the role of GRα and GRβ as markers of corticosteroid-insensitivity in nasal polyposis cannot confirm this finding (112), ultimately leading to the conclusion that neither the GRα receptor, nor GRβ are responsible for glucocorticoid sensitivity in nasal polyposis.
Based on the data above, further studies will be necessary to clarify the various degrees of sensitivity to topical steroids and for a better comprehension the therapeutic effect should be valuated differentiating allergy sufferers with eosinophil polyposis and allergy sufferers without eosinophil polyposis (92,93,116).

Allergic rhinitis and obstructive sleep apnoea syndrome

Obstructive sleep apnoea syndrome (OSAS) is a clinical condition characterised by recurrent nocturnal episodes of apnoea ed hypopnoea, experienced by 4-10% of men and 2-4% of women (117,118).
The physiopathological mechanisms underlying OSAS are complex and have yet to be fully understood (119).
There are two types of relationships that can occur be-

tween allergic rhinitis and OSAS: purely mechanical or inflammatory, both local and systemic.
Interaction between nasal and oral resistance could be implicated in the physiopathology of this disorder. Indeed, the upper airways are described as a Starling resistor which resembles an empty tube partially occluded at the start of the nose area and partly collapsed at the section below it corresponding to the oropharynx (120). The clinical significance of the properties of the resistor imply that changes in pressure and intraluminal resistance and/or the collapsibility of the airways influence patency, so that high inspiratory pressure will reduce airflow (121,122).
Among the factors that influence maximal airflow in the collapsible section of the airways (122) besides increased intranasal pressure, which occurs during therapy (CPAP, BiPAP), or greater collapsibilty due to an anatomical predisposition of the intermediate region of the resistor (oropharynx), a possible third factor is upper airway resistance, particularly in the nose (123).
In the presence of nasal obstruction, the ability of the section below to collapse increases (119) in that a Bernoulli-type effect occurs due to increased negative pressure at the oropharyngeal level during inspiration (124).
The Starling resistor theory therefore hypothesises that nasal obstruction plays an important role in the physiopathology of OSAS (119), though the success rate of corrective nasal surgery is poor, only rarely substantially reducing the frequency of apnoea/hypopnoea attacks (125).
On this point, data is largely controversial regarding nasal obstruction as a risk factor for OSAS. On the one hand studies done using objective parameters of nasal resistance in snorers have been unable to produce a correlation between increased resistance to airflow in the nasal fossa and OSAS (126-128), while on the other, a subsequent study performed on a large snorer population who underwent posterior rhinomanometry successfully found that nocturnal nasal obstruction is an independent risk factor for OSAS (124).
The authors who used multiple regression analysis demonstrated that nasal obstruction contributes to the development of OSAS in 2.3% of cases, while other noted risk factors such as the distance between the hyoid bone and mandibular plane, BMI, male sex and age contribute 6.2 %, 4.6%, 3% and 1.3% respectively to the variation (124).
In addition, a stronger correlation was found between an increase in nasal resistance when supine and OSAS (129,130). Indeed, nasal congestion increases in the supine body position and worsens during sleep, especially in the case of allergic rhinitis when inflammatory mediators peak in the early hours of the morning which combine with a reduced sympathetic nocturnal tone inducing a corresponding increase in parasympathetic tone which is associated with nasal congestion (131).
Several studies have investigated the relationship between nasal obstruction in allergic subjects and respiratory changes during sleep. In their cohort study of 911 subjects who were given a polysomnograph Young et al. (128) reported substantial respiratory changes during sleep in subjects with symptomatic allergic rhinitis compared to those without nasal symptoms. However, the same authors found no linear correlation between reduced upper airways airflow and the severity of breathing changes during sleep.
As allergic rhinitis produces various types of nasal obstruction it has been the subject of study: micro-awakening associated with breathing disorders in sleep are far more frequent in subjects with seasonal allergic rhinitis than in healthy controls (132,133).
One case control study by Canova et al (134) aimed to evaluate whether atopy to perennial allergens and subsequent allergic rhinitis were risk factors for OSAS. The authors concluded that the prevalence of allergic rhinitis was higher in OSAS subjects than in COPD controls. Therefore the author hypothesized that allergic rhinitis to perennial allergens is a risk factor for OSAS and that treatment of this comorbidity is important in reducing OSAS morbility (134). This is the first study to identify the link between OSAS and perennial allergic rhinitis (134). To support this paper's case we cite the study by McNicholas et al. (132) which documents that seasonal allergic rhinitis patients have a higher apnoea/hypopnoea index (AHI) and more protracted seizures of sleep apnoea in the pollen season. More recently, the same author (135) evaluated the efficacy of intranasal steroid therapy in subjects with OSAS and allergic rhinitis by applying the parameters of nocturnal apnoea severity, sleep quality, snoring and daytime symptoms. The OSAS was found to improve, as indicated by a fall in the apnoea/hypopnoea index, with no snoring improvement, leading to the author's conclusion that steroid therapy could benefit selected groups of patients with OSAS.
Subsequent studies reported significant associations between breathing disorders during sleep and nasal obstruction of diverse origins (134,136,137).
As for inflammatory factors in OSAS, it has recently been reported that the presence of systemic inflammation is associated with daytime sleepiness and an increased risk for

cardiovascular complications or metabolic syndrome in these patients (138).
The underlying inflammation of OSAS has been attributed to a mechanical change in airways tissue induced by repeated trauma from snoring and the hypoxia-normoxia cycle of the disorder (138).
Systemic inflammation in OSAS is characterised by an increase in TNF-α, IL-6, PCR, IL-1, and the ICAM-1 adhesion molecule plasma values (139, 140). Intermittent hypoxemia can also stimulate transition factors like nuclear factor-kB and increase cytokine production (141).
A comparison of nasal lavage in OSAS and normal subjects yields a higher number of neutrophils and, concentrations of bradykinins and VIP in the former (142).
The evaluation of inflammation through analysis of induced sputum confirms the high percentage of neutrophils and reduced number of macrophages in OSAS sufferers, while other cell populations do not appear to present any differences from those of healthy controls (143). IL-6 and 8-isopentane values measured in exhaled breath condensate were elevated in OSAS patients compared to those of obese and control subjects (144).
These data demonstrate that similarly to systemic inflammation in OSAS patients, the airways are also characterised by local inflammation and oxidative stress (138).
As previously mentioned, through the hypothetical resistor model the mechanics of the upper airways determines the significant role of nasal obstruction (119). A recent editorial by (119) McNicholas et al. makes the assumption that variable nasal obstruction plays a more important role in the physiopathology of OSAS than anatomical obstruction in the nasal cavities. This is a partially biased point of view which bases itself on the hypothesis that in subjects with fixed nasal obstruction an oral respiration adaptive response develops which limits the impact of the pathogenesis of nasal obstruction OSAS. The response does not seen to occur in intermittent nasal obstruction, whose relationship is more closely linked with automatic nasal respiration.
In children, the prevalence of OSAS is 2-3% (145) of which the highest incidence is between 2-8 year olds. Though anatomical and neuromuscular abnormalities can contribute to the development of OSAS its severity is especially related to adenoid and palatine tonsil size (146, 147) to such an extent that the choice treatment is an adenotonsillectomy (148).
Adenotonsillar hypertrophy is frequent in children with allergic rhinitis (149,150). A study of 28 children with seasonal allergic rhinitis revealed a significant increase in adenoid size in 71% of cases with an associated decrease of the nasopharyngeal cavity in 93% of subjects during the pollen season (151). These changes subside in 90% of the subjects after the pollen season ends.
As a result, in children, snoring and OSAS are associated with adenotonsillar hypertrophy and chronic rhinitis, and allergic rhinitis increases the risk for OSAS especially in children with habitual snoring (152).
The combination of steroid therapy and adenoidectomy is a key competitor in the treatment of paediatric populations (153). However, in the management of OSAS the benefits of oral corticosteroid therapy do not appear to be diminished when compared to the outcome of an adenoidectomy (153), practised by general consensus in the last decade to reduce the size of upper airways lymphoid tissue (154-158).
More recent studies (159) have demonstrated that the upper airways lymphoid tissue in children with OSAS presents a large number of glucocorticoid receptors α, and hypothesise that the better topical steroid therapy response is linked to this circumstance.
In one study (145) of 62 children polysomnographically diagnosed with mild OSAS, the administration of intranasal corticosteroids seemed to reduce the severity of OSAS and the size of the underlying adenoid hypertrophy, which lasted eight weeks after discontinuing therapy. Moreover, in a previous study, after six weeks of topical fluticasone treatment, the steriod improved the apnoea/hypopnoea index (AHI) in children with OSAS (155), which was confirmed by a fall in the number of episodes of haemoglobin desaturation, activation of the respiratory muscles and night time awakenings.

Conclusions

Though allergic rhinitis, viral exacerbations, chronic rhinitis with polyps and OSAS have many inflammatory mechanisms in common, further studies will be necessary to investigate the most controversial hypotheses.
Viral exacerbations in particular belong to this category, where a dynamic relationship and counter relationship seem to develop between the load and nature of the viral infection on one hand and widespread and pre-existing allergic inflammation on the other. That being so dynamism related therapy, Any data remains necessarily undefined regarding the effects of steroid therapy, when there is a concomitant viral inflammatory response of the airway mucus.

Once again, if chronic rhinosinusitis with polyps and allergic rhinitis present an inflammatory cell and cytokine in pictures which match, the etyiological relationship between the two conditions appears to be influenced by the type of antigenic stimulus, which is more powerful in the case of the Staphylococcus aureus super antigens and mycophytes but less for dust mites, pollens and animal dander.

No one questions the efficacy of topical nasal steroids for the treatment of allergic rhinitis especially when obstructive and of eosinophilic chronic rhinitis with polyps.

Data from the literature seem to indicate that nasal obstruction is probably a smaller risk factor for OSAS that other already detected risk factors, and that allergic rhinitis can influence the presence of OSAS through both obstructive and inflammatory mechanical factors. It seems probable that OSAS itself can contribute to the severity of allergic rhinitis. Topical corticosteroid therapy, even in paediatric age is a promising candidate as a new therapeutic tool able to improve symptoms and quality of life in patients with obstructive allergic rhinitis and OSAS

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The impact of air pollution on hospital admission for respiratory and cardiovascular diseases in an oil and gas-rich country

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Key words

Epidemiology. air pollution, PM10, SO2 and O3 pollutant, Qatar, respiratory and coronary heart diseases

Summary

Objectives: *Aim of this study was to evaluate the impact of air pollution on hospital admissions for respiratory and cardiovascular diseases in an oil rich developing country, State of Qatar.* ***Methods:*** *A prospective cohort population based study was conducted at different stations of Qatar during the period (2002-2005) for recording the concentration of air pollutants daily for sulphur dioxide (SO2), nitric oxide (NO), nitrogen dioxide (NO2), carbon monoxide (CO), ozone (O3) and particulate matter (PM10). Hospital admission data were collected from the inpatient discharge database of the Medical Records Department, Hamad General Hospital.* ***Results:*** *An average of 5.36 admissions from ischemic heart diseases was counted daily in all the population which was even higher than the respiratory diseases (3.4/day). Minimum temperature was inversely correlated with all pollutants except for O3 and SO2.* ***Conclusion:*** *There was an association between increasing air pollutant levels and patients admitted for respiratory and cardiovascular diseases.*

Introduction

Air pollution and its public health impacts are drawing increasing concern from the environmental health research community, environmental regulatory agencies, industries as well as the public. The quality of the air, both indoors and outdoors, is closely related to morbidity and mortality from respiratory and cardiovascular diseases. Air pollution is composed of many environmental factors which include Carbon Monoxide (CO), Nitrates, Sulphur dioxide (SO2), Ozone (O3), lead, tobacco smoke and Particulate Matter. Urban atmospheric pollution has a well-known impact on acute and chronic respiratory disease (1). The United Nations estimated that over 600 million people in urban areas worldwide were exposed to dangerous levels of traffic generated air pollutants (2). Air pollution and its impact on human health have been considered a serious problem in urban areas.

At the present time, motor vehicle emissions are the main source of urban pollution than other sources such as heating and industrial activities. Daily levels of air pollutants have been associated with increased daily mortality and morbidity. The time series studies in North America have indicated that particles and ozone are related to emergency hospital admissions for respiratory conditions (3).

Some studies showed strong correlations between air pollutants levels and causes of morbidity with respiratory and cardiovascular diseases (4-11). Also, a study reported as-

sociation between ambient carbon monoxide levels and hospitalizations for congestive heart failure in the elderly in (10) Canadian cities (12,13), air pollution and hospital admissions for cardiovascular disease in Tucson14 and stroke in Kaohsiung, Taiwan (15) and effects of temperature and air pollutants on cardiovascular and respiratory diseases for males and females older than 65 years of age in Tokyo (16). There is substantial epidemiological evidence indicating a link between respiratory and cardiovascular morbidity and outdoor air pollution levels.

Air pollution in the State of Qatar originates mostly from motor vehicle traffic and industry. As a result, concentrations of CO, NO2, O3, and airborne particles are generally high. Expanding industrialization and increasing traffic volumes in the developing countries will drastically increase total emissions of many air pollutants as has been predicted by a study in East Asian Country (17). Hence, the present study was designed to investigate the air pollution and evaluate the impact of air pollution on hospital admissions for respiratory and cardiovascular diseases in Qatar.

Subjects and methods

This is a prospective cohort population based study aiming to investigate the air pollution and the impact of air pollution on respiratory and cardiac diseases in the State of Qatar during the period (2002 – 2005). The State of Qatar is located halfway along the western coast of the Arabian Gulf. The length of the peninsula from south to extreme north is about 160 km, and the total area including the islands is about 11493sq.km. The population estimate of Qatar for the year 2005 was 796186. Doha is the capital and commercial centre of the country. Mesaieed as the second important town is a modern industrial town. Qatar is characterized by a hot summer starting from June till August. Winter is warm with little rainfall. It starts from December to February; Spring starts from March to May; and Autumn starts from September to November.

Data on Air quality and weather

Data on six air pollutants CO, NO2, NO, O3, SO2, PM10 were obtained from the Environmental health department of the Qatar Petroleum. There were stations for monitoring general air quality across the territory and we have taken the readings from the important stations of the urban areas. The hourly concentration record of each air pollutant from each individual station was retrieved during the period 2002 – 2005 and the daily mean of each air pollutant was calculated. Pollutant concentrations are obtained from 24-h average (starting at 4:00 P.M. of the preceding day). Meteriological data including temperature and humidity were obtained from the department of Meteorology, Civil Aviation Department.

Hospital admissions data

In the State of Qatar, there are five government hospitals under the umbrella of the Hamad Medical Corporation (HMC) managing 1567 beds and accounts for 90% of all hospital admissions. These hospitals provide in-patient services for all residents of Qatar and are the main tertiary care centers in the country making an ideal center for population-based studies. All hospital inpatient data including demographic characteristics, dates of admission and discharge, diagnoses and procedures on discharge using the International Classification of Diseases, 9th Revision, Clinical Modification (ICD-9-CM), have been stored in a central-computerized database in the Medical Records departments. We have retrieved the data on monthly hospital admission during the study period for respiratory diseases including Pneumonia and Asthma [(460-466), (480-486), (490-492), & (493-496)], Ischemic heart disease (410-414) and Cardiovascular illness [(420-438) & (440-444)].

Statistical Analysis

Student-t test was used to ascertain the significance of differences between mean values of two continuous variables and confirmed by non-parametric Mann-Whitney test. One-way analysis of variance (ANOVA) was employed for comparison of several group means and to determine the presence of significant differences between group means of continuous variables. Multiple regression analysis was used to assess the relationship between the dependent variable and independent variables. Pearson's bivariate correlation was utilized for association between continuous variables. The level $p<0.05$ was considered as the cut-off value for significance.

Results

Table 1 shows the summary of environmental variables and daily hospital admissions during the study period (2002 – 2005). A daily average of 3.4 respiratory diseases, 3.53 cardiovascular diseases, 5.36 Ischemic heart

diseases were observed in the study period. An average of 5.36 admissions from Ischemic heart diseases was counted daily in all the population which was even higher than the respiratory diseases (3.4/day).

Table 2 presents the day-to-day correlation between Air Pollution and Meteorological Measures. The concentrations of CO and NO2 were highly correlated with other pollutants. PM10 and SO2 were weakly correlated with other pollutants. Minimum temperature was inversely correlated with all pollutants except for O3 and SO2. But, humidity was inversely correlated with all the air pollutants except for NO and NO2. The critical air pollutants in urban areas of Qatar were CO and NO2.

Table 3 presents the effect of meteorological factors on the air pollutants in Qatar. There was a highly significant association between meteorological factors and air pollution.

Table 4 shows the trend in concentration of air pollutants and the number of daily admissions from respiratory and cardiovascular diseases during the study period 2002-2005. As there was a slight increase in the concentration of air pollutants in the year 2005, the daily admissions from the respiratory, Ischemic heart diseases and cardiovascular diseases also increased slightly.

Table 1 - Summary of environmental variables and daily hospital admission from the respiratory and cardiovascular diseases 2002-2005

Variables	Daily Mean	Percentiles			Maximum
		25th	50th	75th	
Pollutant variable					
CO [ppm]	1.01	0.65	0.87	1.10	5.04
NO2 [ppm]	0.033	0.022	0.030	0.039	0.111
NOx [ppm]	0.019	0.003	0.009	0.023	0.120
O3 [ppm]	0.027	0.015	0.027	0.039	0.098
SO2 [ppm]	0.005	0.002	0.004	0.041	0.113
PM10 (Ìg/m3)	98	47	73	264	495
Environmental variable					
Temperature [C]	26.25	20.60	26.90	31.70	46.85
Humidity [%]	59.58	47.55	64.00	75.70	86.40
Hospital admissions (All ages)					
Respiratory	3.40	4.79	6.21	6.93	4.82
Cardiovascular illness	3.53	3.29	3.54	3.80	3.88
Ischemic Heart diseases	5.36	5.16	5.29	5.61	5.74

Discussion

Exposure to air pollution has been considered to be one of the leading factors in public health problems in developing and oil-rich developing countries. This problem has long been the focus of attention in developed countries and their exposure rates have been greatly reduced whereas, relatively, there has not been much effect in reducing the magnitude of the problem in oil-rich developing countries. Documentation of air pollution and sources in the State of Qatar has never been reviewed in terms of its effect on health. No research by means of a population-based study has been conducted in order to define the important epidemiological characteristics of air pollution in human health.

Many authors have reported the effects of air pollutants on the cardiovascular system (4, 16). Carbon monoxide

Table 2 - Day-to-day correlation between Air Pollution and Meteorological Measures

Variable	CO	NO2	NO	O3	SO2	PM10	Tmin	Hmed
CO	1.00							
NO2	0.882*	1.00						
NO	0.679*	0.385*	1.00					
O3	0.378*	0.380*	0.283*	1.00				
SO2	0.581*	-0.350*	0.270†	0.213*	1.00			
PM10	0.753*	0.726*	-0.190	0.132†*	0.054	1.00		
Tmin	-0.191*	-0.264*	-0.030†	0.274*	0.111†	-0.219†	1.00	
Hmed	-0.182*	0.234*	0.031†	-0.323*	-0.228*	-0.317*	-0.663*	1.00

Tmin: Minimum temperature; Hmed: Relatively humidity
†p<0.05
**p<0.01*

Table 3 - The effect of meteorological factors on the air pollutants in Qatar

Pollutants	Meteorological factors				Sig. of F	R-sq
	Wind Speed	Wind direction	Temperature	Relative humidity		
SO2	0.001 (-4.6)*	NS	0.013 (-2.6)	0.001 (-4.2)	0.001	58.2%
NO	0.001 (-5.9)	NS	0.001 (-3.7)	NS	0.0001	75.8%
NO2	NS	0.002 (3.3)	0.001(-4.1)	0.019 (-2.5)	0.0001	69.4%
O3	0.001 (3.7)	0.007 (2.9)	0.001 (-3.9)	NS	0.0001	67.2%
CO	0.001 (-5.4)	0.001 (4.2)	NS	NS	0.0001	67.1%
PM10	NS	0.025 (2.6)	0.002 (3.4)	NS	0.0001	73.6%

* *Significance p–value and Student t–test*
NS = Not –significant
† One–Way Analysis of variance and p–valu

Table 4 - The trend in concentration of air pollutants and the number of dailyadmissions from respiratory and cardiovascular diseases, 2002 - 2005

Variables	Yearly average			
	2002	2003	2004	2005
Air pollutants				
CO	1.070	1.050	1.13	1.19
NO2	0.027	0.030	0.032	0.033
NO	0.013	0.015	0.028	0.029
O3	0.028	0.025	0.027	0.029
S02	0.004	0.004	0.005	0.006
PM10	91.00	99.00	105.00	111.00
Hospital Admissions				
Respiratory diseases	4.428	5.121	5.064	5.300
Cardiovascular diseases	3.419	3.368	3.400	3.914
Ischemic Heart diseases	5.218	5.359	5.247	5.599

(CO) is a well recognised cardiovascular toxicant and its association with the exacerbation of Angina and Myocardial infarctions has already been reported (12, 18, 19). Another study reported that in Hong Kong and London SO2 was associated with increases in cardiovascular disease hospital admissions (8). An increase in SO2 was associated in more than one-third of the studies with increased hospital admissions for myocardial infarction, angina, or Ischemic heart disease (9). Also, the effects of particulate and gaseous air pollution on cardio respiratory hospitalizations was reported by Burnett et al. (20). It was found from our data that an average of 5.36 admissions from Ischemic heart diseases was counted daily during the study period. Daily admissions of other cardiovascular diseases were 3.53 admissions. Also in our study, the data revealed that as the concentrations of CO, SO2, increased in the year 2005, the admission of Ischemic and cardiovascular diseases also increased slightly in the same year.

CO and NO2 have become a major air pollution problem in the urban areas of Qatar, resulting from, ongoing construction nearby, demolition activities, busy traffic, atmospheric chemical reactions, sea spray, and wind-blown sands. The air pollution data in the present study showed that the concentration of CO and NO2 was highly correlated with other pollutants. But, PM10 and SO2 were weakly correlated with other pollutants. This is in consistent with the results reported by Burnett et al. (20) in ten Canadian Cities. In contrast, in south Boston, the concentrations of PM2.5 and PM10 were highly correlated. CO and NO2 were moderately correlated with PM10 (21).

Among the gaseous pollutants, NO2 and O3, which are powerful oxidating agents, may also trigger an inflammatory pulmonary, then systemic reaction with an increase of blood coagulability and platelets (22, 23). The data showed that as the concentrations of NO2 and O3 increased, there was an increase in the number of admissions from respiratory diseases. This shows a positive association between air pollution and respiratory diseases, as has been reported elsewhere (24). These findings are supported by similar associations between hospitals admissions for respiratory diseases and mortalities that have been reported in a study done in China (9).

This study indicated the importance of following points: Developing a new control strategy to manage and improve air quality. Consideration should be given to the fu-

ture expansion of towns and cities towards industrial emission sources, and the potential effect on air quality of residential areas as a possible consequence. Enforcement of legislation and punishment of polluters is important according to type and intensity of pollution. It is good to establish educational programs for factory managers and workers to increase air pollution awareness. Furthermore, forming collaboration between the Municipality, Environmental Protection Agency and the National Health Authorities with regard to the effect of air pollution on human health is important for the future safety.

Conclusions

The present study findings showed that there was a significant association between the pollutants and meteorological conditions. Also, an association was found between increasing pollutant level and patients admitted for respiratory and cardiovascular diseases. Results showed that the critical air pollutants in the urban areas of Qatar were CO and NO2.

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A murine model of cow's milk protein-induced allergic reaction: use for safety assessment of hidden milk allergens

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Key words

Cow's milk allergy, anaphylaxis, mouse model, LOAEL, NOAEL, margarine

Summary

Background: *Masked allergens in processed food products can lead to severe allergic reactions following unintentional ingestion. We sought to develop a murine model for the detection of hidden cow's milk proteins (CMP). This study aimed to induce cow's milk allergy in mice, to characterize the anaphylaxis induced by CMP in this model, and to validate its reliability using three margarines manufactured with (A) or without (B, C) milk, sharing the same production line.* ***Materials and Methods:*** *Three-week-old BALB/c mice were sensitized intragastrically with CMP plus cholera toxin and boosted 6 times at weekly intervals. CMP-sensitization status was monitored by skin tests, and measurement of CMP-specific IgE and IgG1 levels. On day 44, the minimal threshold of clinical reactivity to CMP in terms of anaphylaxis was determined by performing a dose response of intraperitoneal CMP challenge. Under the same conditions, anaphylaxis was evaluated in CMP-sensitized mice after challenge with protein extracts of margarines A, B or C.* ***Results:*** *Sensitization to CMP was demonstrated by positive skin tests and increased CMP-specific IgE and IgG1. The minimal clinical reactivity threshold corresponding to 0.1 mg CMP elicited detectable anaphylaxis evidenced by clinical symptoms, a decrease in breathing frequency, and increased plasma histamine upon challenge. Similarly, challenges with margarine A containing CMP demonstrated anaphylaxis, whereas those with B or C did not elicit any detectable allergic reaction.* ***Conclusion:*** *This study shows that our murine model of CMP-induced anaphylaxis is useful for investigating the allergenic activity and the assessment of margarines with respect to milk.*

Abbreviations: CMP: cow's milk proteins; CT: cholera toxin; i.g.: intragastrically; i.p.: intraperitoneal; LOAEL: lowest observed adverse effect level; MOS: margin of safety; NOAEL: no-observed adverse effect level

Background

Food allergy is an important public health problem in industrialized countries. To date, strict dietary avoidance is the only way to manage food allergy, which implies careful labelling of manufactured products. Nevertheless, masked allergens in food can lead to severe accidents such as fatal food-induced allergic reactions following unintentional ingestion [1]. The total absence of any or all allergen in foods is often difficult to achieve because of manufacturing practices. Any remaining allergens are due primarily to different products sharing the same production line.

The relationship of any given food to allergy can be considered as two main components. 1) Allergenicity is defined as the likelihood of a given protein to induce *de novo* sensitization in a non-allergic individual [2]. The determination of allergenicity requires models of allergic sensitization primarily conducted in animals. Several models developed in mice [3-8] and rats [5, 9, 10] have been helpful in investigations of allergic sensitization and humoral immune responses. 2) Allergenic activity reflects the propensity of a substance to induce allergic reactions in sensitized individuals [2]. This activity is usually evaluated in allergic patients by oral challenge tests. The clinical objective is to determine whether the component in question induces allergic response in allergic individuals and to estimate the magnitude and the risk related to this reaction. Several rodent [11-13] and non-rodent models such as swine [14] or canine [15] have been developed to mimic food allergies similar to those seen in humans. A major advantage of these models is that a protein induces not only an immune response but, also clinical symptoms as well after allergenic challenge in sensitized animals. These models are useful for the investigation of allergenic activities of allergens and the immunopathological me-chanisms involved, as well as for the exploration of potential immunotherapeutic approaches. Despite these interesting and valuable models, none have been used for the study of the allergenic activity of finished food products before their marketing. *In vitro* assays are commonly used to detect proteins in food products [16-21]. These tests provide information for safety assessment, but do not determine the allergenic activity of finished products. It is clear that clinical studies are the gold standard tests, but in practice, they cannot be implemented on a routine basis for detection of allergens in foods. Genetically Modified Foods by the Food and Agricultural Organization of the United Nations (FAO)/World Health Organization (WHO) [22, 23] has identified a need for the development of well-defined food allergy animal models that can serve as predictive tools for the determination of the allergenic activity of finished food products.

Cow's milk allergy is one of the most common food allergies in infants. Most patients outgrow this by the age of 5 years, but cow's milk allergy can persist in some adults [24]. Contamination of food products with milk proteins have been reported to be unsafe in children allergic to cow's milk [1, 25]. The wide use of cow's milk proteins (CMP) in various food products complicates the application of dietary avoidance. This is most notably the case for fats used as cooking oils or spreads such as margarines. These products are defined as foodstuffs other than butter whatever their origin or their composition, that present the same aspect as that of butter and are intended for the same use. Margarines are composed of two major fractions: fat (83 %) and an aqueous fraction (17 %) which includes water and/or milk, emulsifiers, conservatives, aromas and coloring agents. Some margarines therefore contain cow's milk allergens when milk is included in their manufacturing, while others prepared without milk can be contaminated due to manufacturing practices.

This study aimed (i) to induce cow's milk allergy in mice and characterize the anaphylactic reaction induced by CMP in this model, and (ii) to validate the suitability and the reliability of this model for the testing of margarines manufactured with or without milk, yet sharing the same production line.

Materials and Methods

Three-week-old female BALB/c mice were purchased from Charles River Laboratory (Lyon, France). Animals were maintained on milk-free chow (Harlan Teklad, Gannat, France) under specific pathogen-free conditions on a 12 h light/dark cycle in a room maintained at a mean temperature of 21 ± 2°C with a relative humidity of 50 ± 20%. Drinking water and standard laboratory animal food pellets were provided *ad libitum*. Animals were handled in accordance with French State Council guidelines for the use and care of laboratory animals (decree N° 87-848, October the 19, 1987 and decree 2001-464, May the 29, 2001).

Commercially available powdered cow's milk (355 mg CMP/g, Régilait, Saint-Martin-Belle-Roche, France)

was used. Three margarines referred to as A, B and C were provided by a manufacturer without any indication on their composition. Detection antibodies for ELISAs, i.e. HRP-labeled goat anti-mouse IgE and IgG1, were purchased from Serotec Ldt (Kidlington, Oxford, UK) and Southern Biotech (Southern Biotechnology Associates Inc., Birmingham, AL, USA), respectively. Compound 48/80, red blood cell lysis buffer and concanavalin A were obtained from Sigma (Saint Louis, MO, USA).

BALB/c mice were sensitized intragastrically (i.g.) with cow's milk administered together with cholera toxin (CT) and boosted 6 times at weekly intervals. To determine the optimal sensitizing dose, 3 groups of mice received 0.1, 1 or 10 mg of CMP in PBS containing 4 μg CT per mouse (200 μL per mouse) through oral administration. Control mice were sensitized i.g. with 4 μg CT alone. Naive mice never exposed to CMP or CT were used as second controls. Immediately prior to each boosting, individual blood samples from each group of mice were obtained from the retro-orbital venous plexus under isoflurane anaesthesia, centrifuged and the sera were stored at - 20°C until use. Two skin tests were performed: an ear swelling test and an intradermal skin test (see below). Forty four days after the initial boosting, mice were challenged intraperitoneally with 15 mg CMP in 150 μL of PBS per mouse, and anaphylaxis was assessed by monitoring clinical symptoms, rectal temperature, breathing frequency, and by measuring plasma histamine levels.

Ear swelling test was performed as previously described (Proust et al., 2008). Briefly, CMP (10 μL, 5 mg/mL) was intradermally injected into the dorsal aspect of a mouse ear and ear thickness was measured with a digimatic micrometer (Mitutoyo, Japan). Ear swelling response was determined as the incremental increase in thickness above baseline control values. Compound 48/80 (5 mg/mL) and PBS were used as positive and negative controls, res-pectively.

Intradermal skin tests were carried out as previously described (Proust et al., 2008). Briefly, before testing, the abdominal skin was shaved. Evan's blue dye (100 μL, 0.25 %) was intravenously injected and five minutes later, CMP (10 μL, 2.5 mg/mL) was injected intradermally under isoflurane anaesthesia. Compound 48/80 (30 μg/mL) and PBS were used as positive and negative controls, respectively. A blue wheal with a diameter > 0.3 cm appea-ring within 5 minutes after the injection of allergen was considered as positive.

CMP-specific antibodies were assayed by ELISA. Plates (MaxiSorp, Nunc Immunoplate, Roskilde, Denmark) were coated overnight with CMP (0.5 μg/mL for specific IgE and 1 μg/mL for specific IgG1) diluted in carbonate buffer (50 mM, pH 9.6). Plates were incubated with diluted serum samples (1:10 for IgE; 1:5000 for IgG1) at 37°C for 2 h. CMP-specific IgE were detected by HRP-labeled goat anti-mouse IgE (1:5,000). CMP-specific IgG1 were detected by HRP-labeled goat anti-mouse IgG1 (1:1,000). Plates were developed with tetramethyl benzidine substrate (Pierce, Rockford, IL, USA) and read at 450 nm with an automated microplate reader (Biorad, Hercules, CA, USA). The specificity of HRP-labeled goat anti-mouse IgE was verified in preliminary experiments. IgE detection was not modified after removing IgG from mouse pooled sera with protein-G (Sigma) (data not shown).

Anaphylactic symptoms were assessed by 2 independent investigators within 0-45 minutes after the intraperitoneal (i.p.) challenge; this study was conducted in a blind manner. Disease severity was evaluated by using a scoring system as previously described (Proust et al., 2008) with slight modifications and scored as follows: 0, no symptoms; 1, reduced activity; 2, scratching and rubbing around the nose, the ears and eyes, partial immobility; 3, prostration, pilar erection, total immobility; 4, edema around the mouth and the eyes, puffiness around the eyes; 5, no activity after prodding, convulsion, and death.

Rectal temperature was measured before and 30 minutes after the i.p. challenge using a thermal probe (Anritsu meter CO., LTD, Tokyo, Japan).

Breathing rate (breaths per minute, bpm) was assessed in conscious unrestrained mice following evaluation of anaphylactic symptoms after the i.p. challenge using a barometric plethysmography method (EMKA Technologies, Paris, France).

Blood was collected 60 minutes after the i.p. challenge and plasma histamine concentrations were measured with an ELISA kit (Immunotech, Marseille, France) according to the manufacturer's instructions.

Spleens were harvested from mice allergic to CMP after challenge under sterile conditions. After lysis of red blood cells with buffer (Sigma) and several washes, splenocytes were resuspended in complete culture medium (RPMI-1640 plus 10 % fetal calf serum, 1 % penicillin/streptomycin and 1 % L-Glutamine). Cells were incubated in 24-well plates (4 x 10^6 cells/mL) in the presence or absence of CMP (5 μg/mL) or Concanavalin A (2 μg/mL, positive control) for 72 h at 37°C (5 % CO2). Supernatants were then removed and stored at -

80°C until use. Levels of IL-4, IL-5 and IFN-γ were assayed using CytoSetsTM kits (BioSource International Europe, Nivelles, Belgium) according to the manufacturer's instructions. The limits of detection for IL-4, IL-5 and IFN-γ were < 5 pg/mL, 3 pg/mL and 1 pg/mL, respectively.

To determine the clinical reactivity threshold in CMP-sensitized and -challenged mice, i.e. the minimal dose of CMP leading to anaphylactic symptoms, mice sensitized with the optimal sensitizing dose of CMP as previously determined, as well as CT mice, were blind challenged intraperitoneally at day 44 either with 0, 0.01, 0.1, 1, 5 or 15 mg CMP per mouse.

Protein extracts of each of the margarines (A, B and C) were freshly prepared by treating 10 g melted margarine with di-isopropyl ether. Samples were mixed for 30 min at room temperature on a circular rotator (30 rpm) and then centrifuged 5 min at 2500 rpm. The aqueous phase was collected and organic solvent was evaporated by N_2(g). A negative internal control, i.e. PBS alone, was prepared similarly and simultaneously with the different samples of protein extracts. Mice sensitized to CMP, as well as CT mice were blind challenged by i.p. injection with 150 µL of protein extracts of either margarines A, B or C or with 150 µL PBS alone in order to determine for presence of CMP in margarines.

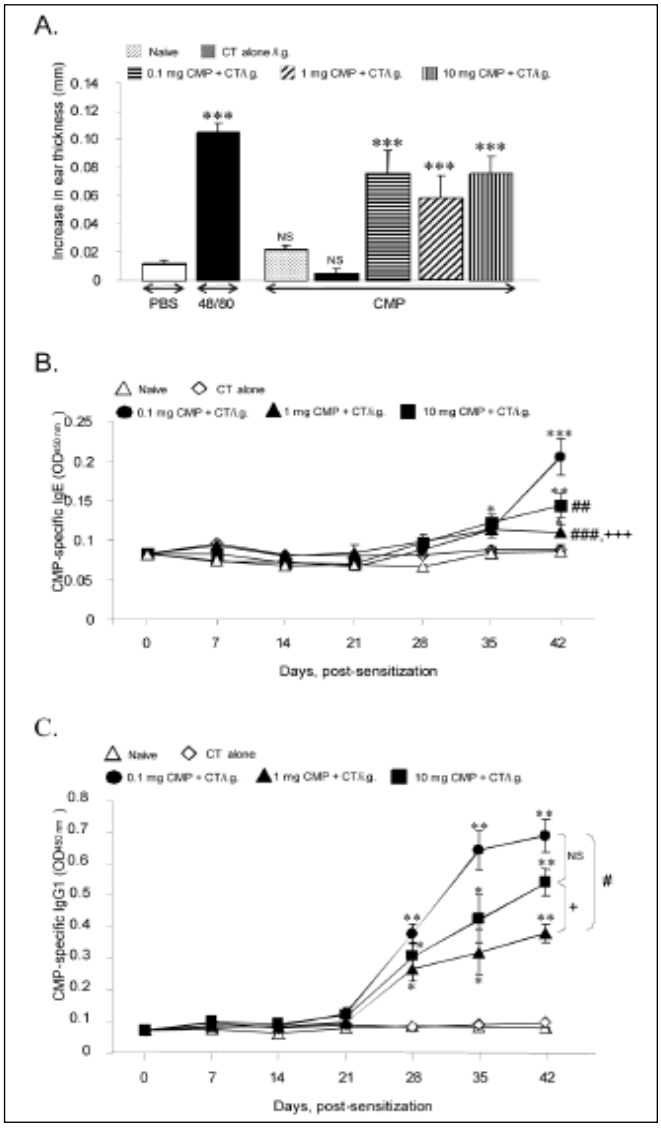
Margarine extracts were prepared as described in the previous paragraph. CMP content of these extracts was measured by sandwich ELISA using polyclonal antibodies directed against all CMP (Neogen, Ayr, Scotland).

Results are expressed as mean ± SEM. Statistical analyses were determined using Student's t test and one-way ANOVA. A p value < 0.05 was considered as statistically significant.

Results

To characterize the relationship between the dose of CMP administered and sensitization status, we performed skin tests (ear swelling and intradermal skin tests) and monitored sera CMP-specific IgE. On day 42, signi-ficant increases in ear thickness in response to intradermal injection of CMP were observed with all sensitizing doses of CMP (Figure 1A). No increase in ear thickness was obtained in control mice (naive and CT alone). Similarly, positive skin responses with intradermal skin test were observed in all CMP-sensitized mice as compared to control mice (data not shown). Animals

Figure 1 - CMP-sensitization following oral exposure to CMP plus CT (A) Ear swelling response after CMP intradermal injection at day 42 post-sensitization. Forty minutes after intradermal injection of PBS, compound 48/80 or CMP (10 µL, 5 mg/mL), increase of ear thickness (mm) was measured in CMP-sensitized mice and control (naive and CT alone). Results are expressed as mean ± SEM of 6 mice per group. ***p < 0.001: CMP or compound 48/80 *versus* PBS treatment for each group. NS: non significant. Sera CMP-specific IgE (B) and IgG1 (C). Pooled sera from each group of mice (n=6 mice/group) as indicated were obtained weekly just before each boosting. CMP-IgE and IgG1 levels were assessed by ELISA. Results are expressed as mean ± SEM of 6 mice per group. *p < 0.05, **p < 0.01, ***p < 0.001: CMP-sensitized *versus* naive or CT mice. #p < 0.05, ##p < 0.01, ###p < 0.001 *versus* 0.1 mg CMP + CT. +p<0.05, +++p < 0.001 *versus* 10 mg CMP + CT. NS: non significant



sensitized with 0.1, 1 and 10 mg CMP plus CT produced significant increase in CMP-specific IgE and IgG1 levels from 35 and 28 days, respectively, after the initial boosting in contrast to control mice (Figures 1B, 1C). On day 42, both 1 and 10 mg doses of CMP plus CT induced an increase in levels of CMP-specific IgE that were significant, but lower than that observed for 0.1 mg CMP plus CT (Figure 1B). CMP-specific IgG1 levels were significantly increased on day 42 at the dose of 1 mg CMP plus CT, but lower than that observed for 0.1 and 10 mg CMP plus CT (Figure 1C).

We next evaluated the anaphylactic reaction in CMP-sensitized mice at day 44 upon an i.p. challenge of 15 mg CMP per mouse. CMP-sensitized mice expressed severe anaphylactic symptoms reaching a clinical score of 4 to 5 irrespective of the sensitizing dose (Figure 2A). However, the dose of 10 mg CMP elicited a more consistent anaphylactic response in all CMP-sensitized mice as compared to those sensitized to lower doses of CMP. In contrast, control mice obtained a clinical score of 0 (Figure 2A). Measurement of changes in body temperature and breathing frequency were consistent with clinical score and provided an assessment of anaphylactic responses that was significantly more pronounced in mice sensitized with 10 mg CMP plus CT (Figures 2B and C). We therefore selected 10 mg CMP plus CT as the optimal sensitizing dose for BALB/c mice. This dose was used in the remainder of the study.

We next determined the production of Th1 and Th2 cytokines by spleen cells stimulated *in vitro* with CMP and collected from BALB/c mice (sensitized with 10 mg CMP plus CT) allergic to CMP. Seventy-two hours post-culture, Th2 cytokine production was significantly increased in CMP-stimulated cultures, 8 ± 0.6 pg/mL ($p<0.01$) and 140 ± 35.8 pg/mL ($p<0.001$) for IL-4 and IL-5, respectively, when compared to unstimulated cells (undetectable). In contrast, IFN-γ levels in CMP-stimulated and unstimulated spleen cells (35 ± 3.4 pg/mL and 31 ± 2.9 pg/mL, respectively) were essentially the same (non significant). IL-4, IL-5 and IFN-γ levels for concanavalin A were 28 ± 1.1 pg/mL, 547 ± 15.4 pg/mL and 695 ± 1.6 pg/mL, respectively.

At day 44, CMP-sensitized BALB/c were challenged intraperitoneally either with 0, 0.01, 0.1, 1, 5 or 15 mg CMP, respectively in order to determine the clinical reactivity threshold. CMP-sensitization status was also confirmed. Dose response curve of Figure 3A shows that a significant increase in anaphylactic clinical score was observed with 0.1 mg CMP challenge and reached a plateau with higher doses. Figure 3 also revealed that a dose of 1 mg CMP induced a maximal decrease in body temperature (Figure 3B) and in breathing frequency (Figure 3C). Although relatively less pronounced, both parameters remained significantly modified at higher

Figure 2 - Anaphylactic response depending on the amount of CMP used for oral sensitization CMP-sensitized (n = 6/group), naive (n = 6/group) and CT (n = 6/group) mice were challenged intraperitoneally with 15 mg CMP at day 44 post-sensitization. (A) anaphylactic symptoms, (B) change in body temperature and (C) change in breathing frequency were evaluated after i.p. challenge. Data are given as mean ± SEM. *p<0.05, **p < 0.01, ***p < 0.001: CMP-sensitized *versus* naive or CT mice after CMP challenge. #p < 0.05, ##p < 0.01 *versus* 10 mg CMP + CT. NS: non significant

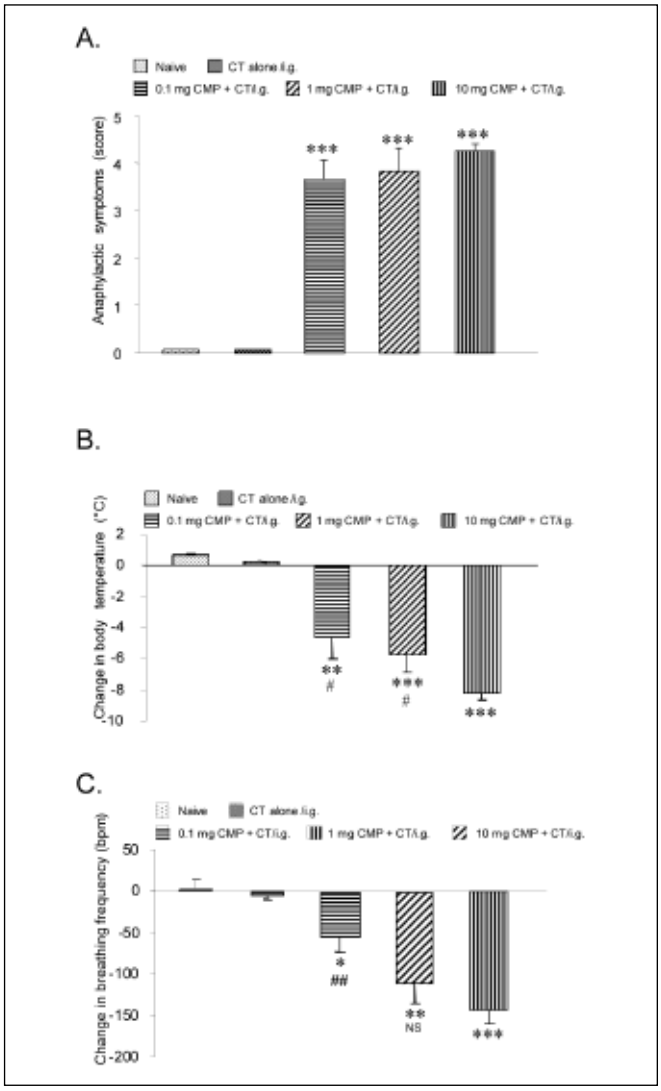
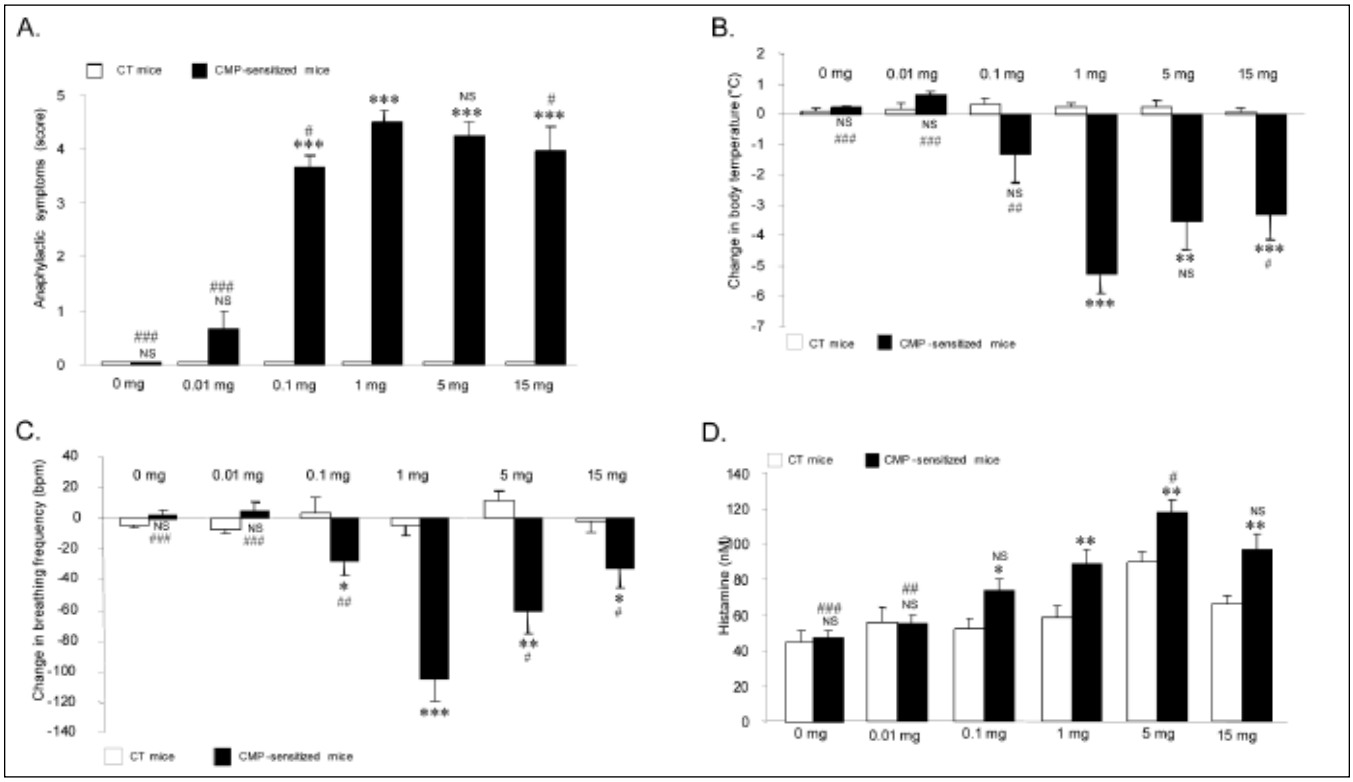


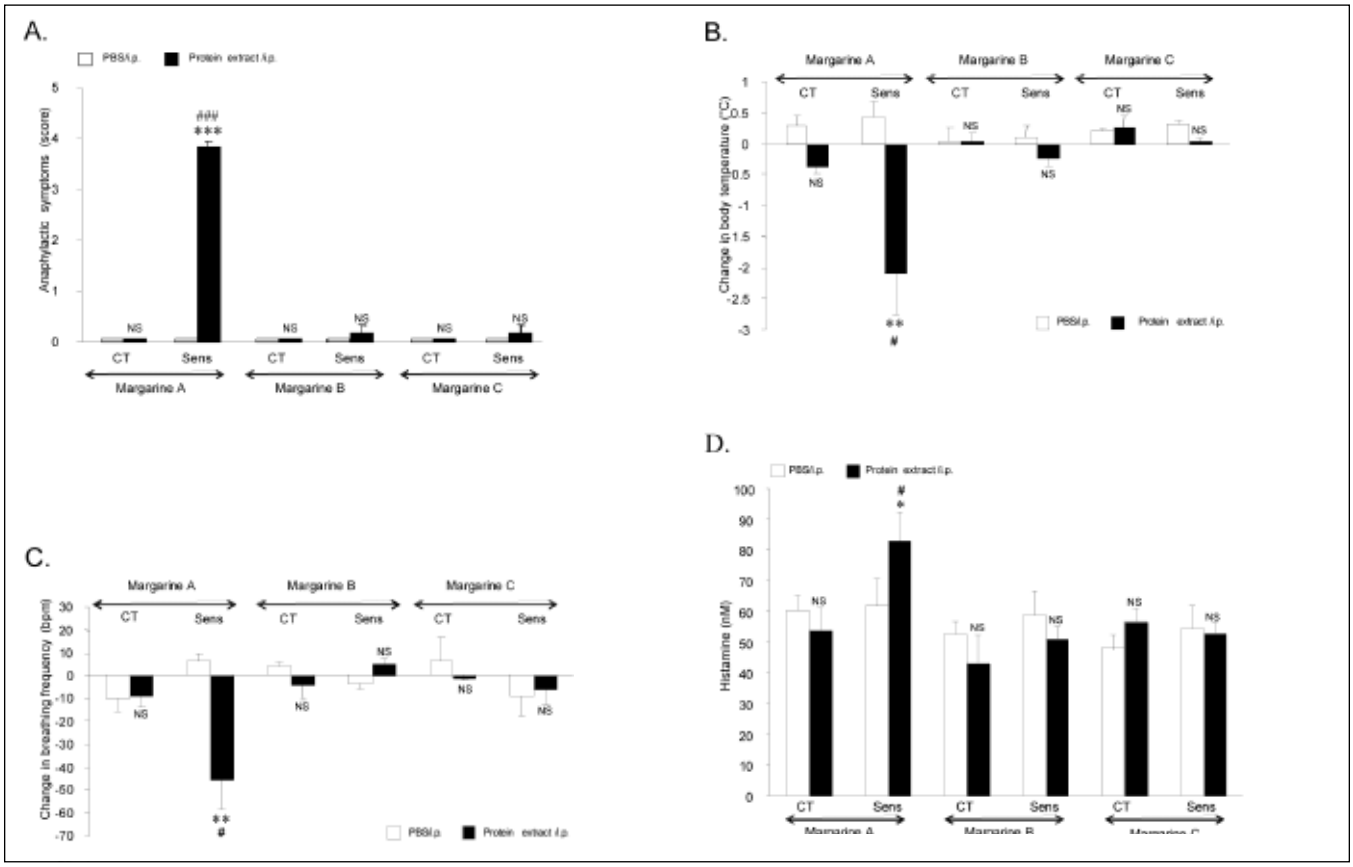
Figure 3 – Threshold of clinical reactivity to CMP in CMP-sensitized mice. Sensitized mice with 10 mg CMP plus CT (n = 6/group) and CT mice (n = 4/group) were challenged intraperitoneally either with 0, 0.01, 0.1, 1, 5 and 15 mg CMP per mouse at day 44 post-sensitization. The clinical reactivity threshold for CMP was determined by monitoring (A) anaphylactic symptoms, (B) change in body temperature, (C) change in breathing frequency and measuring (D) plasma histamine concentrations, after i.p. challenge. Data are expressed as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001: CMP-sensitized *versus* CT mice after CMP challenge. #p < 0.05, ##p < 0.01, ###p < 0.001 *versus* 1 mg CMP. NS: non significant



challenge doses (5 and 15 mg CMP). Histamine release was significantly increased with a challenge dose of 1 mg but further increased with 5 mg CMP (Figure 3D). Therefore, the minimal dose of 0.1 mg CMP elicited clinically detectable allergic reaction. However, a dose of 1 mg was necessary to obtain objective measures of anaphylactic reaction.

BALB/c mice sensitized with 10 mg CMP plus CT leading to positive skin tests and significant increase of CMP-specific IgE in serum were blind challenged intraperitoneally at day 44 either with protein extracts from margarines (A, B, or C) or PBS. CT mice treated either with PBS or protein extracts of margarines did not develop anaphylactic reactions in terms of clinical symptoms, decrease in body temperature and in breathing frequency (Figures 4A, B and C). Among the 3 tested margarines in CMP-sensitized mice, the extract from margarine A led to anaphylaxis with a clinical score in a range of 3 to 4 associated with a statistically significant 1) drop in body temperature, 2) decrease in breathing frequency and 3) release of plasma histamine (Figure 4). Margarines B or C failed to induce any detectable anaphylactic reactions. These results indicated that only margarine A contained CMP in quantity sufficient to provoke an allergic reaction. We next estimated the concentration of proteins in the extracts of margarines A, B and C. Immunobiochemical analysis revealed that CMP concentrations of extracts of margarines A, C and B were 10.5 μg/μL, 0.0035 μg/μL and undetectable, respectively. Consequently, we estimated the quantity of CMP administered intraperitoneally per mouse in a final volume of 150 μL to be 1.6 mg and 525 ng per mouse for margarines A and C, respectively.

Figure 4 *- In vivo* assessment of allergenic activity of margarines. On day 44 post-sensitization, mice sensitized with 10 mg CMP plus CT and CT mice were challenged intraperitoneally either with protein extracts of margarines (A, B, C) (Sens n = 6, CT n = 4 per margarine) or with PBS (Sens n = 6, CT n = 4 per margarine) used as the negative internal control. (A) anaphylactic symptoms, (B) change in body temperature, (C) change in breathing frequency and (D) plasma histamine concentrations were evaluated after i.p. challenge. Data are given as mean ± SEM. *p<0.05, **p<0.01, ***p < 0.001: protein extract *versus* PBS challenge within each group. #p < 0.05, ###p < 0.001: CMP-sensitized *versus* CT mice after challenge with protein extract. NS: non significant



Discussion

This is the first report of the application of animal model towards detection of the allergenic activity of hidden milk allergens extracted from food using a murine model of cow's milk-induced allergy. However, this is not the first description of CMP-induced anaphylaxis. Previous interesting and valuable models of CMP-induced allergy have been reported [12, 27], but none of them have determined the clinical reactivity threshold doses to CMP in order to define the lowest observed adverse effect level (LOAEL) and the no-observed adverse level (NOAEL). Similarly to the experimental approach reported in these studies, mice were sensitized using several oral exposures of milk plus CT. Increased CMP-specific IgE and IgG1 levels and positive skin tests to CMP demonstrated sensitization to CMP in BALB/c mice. In the context of providing a sensitive model for the detection of CMP, the i.p. route was used for allergenic challenge to elicit anaphylaxis [26, 28]. This route offers the advantage of minimizing variations of allergen bioavailability. Indeed, we demonstrated recently that this route was much more sensitive than the i.g. route in terms of anaphylactic response [28]. The determination of the threshold clinical reactivity to CMP is based on the assessment of anaphylactic reaction by monitoring the clinical symptoms and quantifiable parameters (body temperature, breathing frequency, histamine). In our model, the LOAEL was found to be 0.1 mg CMP. This dose was demonstrated to be favourable towards eliciting a detectable allergic reac-

tion including anaphylactic symptoms scored in a range of 3 to 4 associated with a significant decrease in breathing frequency and increased release of plasma histamine compared to 0.01 mg CMP. This latter dose that failed to lead to anaphylactic reaction corresponds to the threshold CMP dose below which no adverse effects occur and thus is defined as the NOAEL in our model [29]. As shown in our study, the 1 mg CMP dose was necessary to obtain objective measures of anaphylactic reaction including body temperature, breathing frequency and plasma histamine release. In case of 0.1 mg CMP dose, no global significant change in body temperature was observed suggesting that the temperature is related to the variability of response in mice. Indeed for this dose, a marked decrease in body temperature was only recorded in some individuals (n = 2 mice/6) indicating that a drop in body temperature is nevertheless a sign of disease severity [30-32]. According to our results, clinical tests were required to evaluate the allergenic activity of milk allergens in terms of anaphylaxis, because they clearly evidenced a severe sign of anaphylactic shock. Moreover, the combination of these clinical tests with a biologic assay such as the measurement of plasma histamine release is important in order to confirm the involvement of mast cells in CMP-specific anaphylaxis.

In practice, the reliability of our model as CMP detection tool was tested by assessing the allergenic activity of 3 different margarines A, B and C sharing the same production line, manufactured with or without milk. Indeed, only CMP-sensitized mice challenged with margarine A exhibited an anaphylactic reaction similar to that observed with CMP challenge, indicating the presence of CMP at levels sufficient to provoke anaphylaxis. On the other hand, no anaphylactic reaction was developed with margarines B and C, suggesting that either the finished margarines did not contain CMP, the concentration of CMP was below the limit of detection, or that the margarines contained proteins without allergenic activity. We then evaluated the levels of CMP in the margarine extracts that led to the appearance of anaphylactic symptoms in order to compare these clinical data to the murine LOAEL or NOAEL. This allowed us to evaluate the feasibility of this model as a tool for determining the allergenic risk of the analyzed margarines. The lack of allergenic activities of margarines B or C was supported by the immunobiochemical evaluation of the CMP amounts in the extracts of margarines. Indeed, in contrast to margarine A (1.6 mg CMP per mouse), under the same conditions, margarine C did not lead to anaphylaxis probably due to the fact that 525 ng CMP per mouse is largely below the LOAEL and the NOAEL, nor did margarine B due to the absence of any detectable protein. The LOAEL described in human varies between 0.6 and 180 mg CMP, whereas no NOAEL for milk has been reported [33]. Interesting and valuable existing *in vitro* assays are more sensitive for protein detection, but their major inconvenience is lack of information on the allergenic activity of food products in contrast to *in vivo* detection tools [16-21]. The application of our model to margarines confirms the fact that this murine model of CMP-induced anaphylaxis may be used as a tool to assess the safety of a finished food product for people with cow's milk allergy. Mice did not exhibit allergic reactions with 525 ng CMP in margarine C, which is 1150 times below the human LOAEL. The harmlessness of the margarine C is confirmed with a margin of safety (MOS) > 100, which is established from the mouse NOAEL [29]. Since a MOS > 100 is considered to be without risk for human subjects [29]. Thus, this margarine could be considered as being of no risk to CMP-allergic patients. CMP detected in margarine C are most likely contaminants resulting from the use of CMP on the same production line. On the other hand, the margarine A would be prohibited to patients allergic to CMP because of the wide overlap between the murine and human LOAEL values. The knowledge of a NOAEL for milk obtained from animal studies could provide the food industry with a much needed MOS to establish good manufacturing practices and allergenic risk control programs. This model could be used as a supplement to the biochemical tests in order to investigate a potential allergenic activity when a biochemical risk with respect to milk has been detected in a food product intended for allergic consumers before its marketing.

Conclusions

We report here the development and characterization of a BALB/c model of CMP-induced anaphylaxis that represents a potential *in vivo* CMP detection tool for the safety assessment of finished food products such as margarines. Additional studies are required to determine the capacity of our model to evaluate the CMP allergenic activity of other finished food products, i.e. to analyze whether the food product contains specific CMP allergens at levels that could potentially induce an allergic reaction in sensitized individuals.

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News

Preventable Injuries from Life-Saving Epinephrine Auto-Injectors on the Rise

ARLINGTON HEIGHTS, Ill., April 6, 2009 – Researchers find an increased rate of unintentional injection of epinephrine from auto-injectors for anaphylaxis (severe allergic reactions) and urge people who may need to administer the life-saving drug to themselves or others in an allergic emergency to receive regular coaching in its proper use. The report is published this month in Annals of Allergy, Asthma & Immunology, the scientific journal of the American College of Allergy, Asthma and Immunology (ACAAI).

More than 50 million Americans suffer from some type of allergy. While an allergy often makes people miserable, it's rarely dangerous, unless it results in an anaphylactic reaction, an allergic emergency. Fast-acting, self-administered epinephrine (adrenaline) auto-injectors are commonly prescribed for people who are at risk of anaphylaxis.

Systematically reviewing 26 reports published in peer-reviewed journals during the past 20 years, F. Estelle R. Simons, M.D., Department of Pediatrics and Child Health, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada, and colleagues in the United States, found that most of the 69 incidents of unintentional injection of epinephrine reported to date in the medical literature have occurred during the past 6 years.

The true rate of occurrence of unintentional injection of epinephrine from auto-injectors is unknown, but the authors note that the previously projected rate of 1 in 50,000 injections has been seriously underestimated.

"An increased rate of occurrence is likely, paralleling the increased rate of occurrence of anaphylaxis in the community and the increased dispensing rates for epinephrine auto-injectors," they stated.

Although approximately 10 percent of the injuries occurred while first aid treatment was being administered to another person, no information about the outcomes of anaphylaxis in the person for whom the epinephrine was intended was found in the articles reviewed.

The researchers therefore note that additional information is needed "about the lost dose hazard and its implications for anaphylaxis morbidity or mortality and about the indications for, and timing of, a second injection of epinephrine in this situation."

Although inadvertent injuries from epinephrine auto-injectors sometimes cause extreme discomfort, they generally have a favorable outcome.

Authors conclude, "Health care professionals should maintain vigilance about training and regular coaching of those at risk for anaphylaxis in the community and the caregivers of children at risk in the correct and safe use of epinephrine auto-injectors, ideally at yearly intervals."

About Anaphylaxis

People who have allergies and/or asthma and a history of severe allergic reaction are at increased risk, but anyone can have an anaphylactic reaction.

The most common triggers of a anaphylaxis are food (especially peanut, tree nuts – almonds, pecans, cashews, walnuts – fish, shellfish, cow's milk and egg), insect stings, medications (most commonly penicillin) and latex. Its symptoms include:

- Hives, itching and redness of the skin, lips, eyelids, or other parts of the body, and/or itching of the throat, tongue, and mouth
- Wheezing and/or difficulty breathing
- Swelling of the tongue, throat and nose
- Nausea, vomiting, diarrhea, or cramping pain in the abdomen
- Dizziness and fainting or loss of consciousness, which can lead to shock and heart failure

Patient information on allergic diseases including the free brochure, titled Be S.A.F.E Managing Allergic Emergencies (Anaphylaxis), is available by calling the ACAAI toll free number at (800) 842-7777 or visiting its Web site at HYPERLINK "http://www.acaai.org" www.acaai.org. For food allergy patient information or support, call the Food Allergy and Anaphylaxis Network (FAAN) at (800) 929-4040 or visit online at HYPERLINK "http://www.foodallergy.org" www.foodallergy.org.

News release issued by the American College of Allergy, Asthma and Immunology (ACAAI)

Caregivers of Asthmatic Children Fail to Use Albuterol Properly

ARLINGTON HEIGHTS, Ill., June 10, 2009 - Nearly one third of caregivers in low-income, urban areas used albuterol improperly in the home when treating children for acute asthma symptoms, according to a report published this month in Annals of Allergy, Asthma & Immunology, the scientific journal of the American College of Allergy, Asthma and Immunology (ACAAI).

Jane M. Garbutt, MB, ChB, FRCP (C), associate professor of medicine and pediatrics, medical director of Washington University Pediatric/Adolescent Ambulatory Research Consortium, Washington University School of Medicine, St. Louis, Mo., and colleagues, report that 32 percent of 114 caregivers in the intervention group of a randomized trial to reduce emergent care for low-income urban children used albuterol inappropriately (over-treatment or under-treatment).

"Albuterol is the most effective treatment for providing prompt relief from worsening asthma systems and is recommended for home use, guided by an asthma action plan," note the authors.

The caregivers completed a structured telephone interview with an asthma nurse to evaluate home management of their child´s acute asthma symptoms. Albuterol use for worsening asthma symptoms was categorized as appropriate for only 68 percent of caregivers, and was more likely if the children had an emergency department visit or hospitalization for asthma in the prior year.

Reportedly having an asthma action plan, or a recent primary care physician visit to discuss asthma maintenance care, did not increase the likelihood that albuterol use was appropriate.

"Caregivers reported that they would use albuterol to treat their child´s worsening asthma symptoms, but many described inappropriate use," the authors conclude. "Detailed evaluation of proper albuterol use at home may provide insight into how health care professionals can better educate and support parents in their management of acute exacerbations and more effective use of asthma action plans."

The National Asthma Education and Prevention Program (NAEPP) guidelines recommend early treatment of acute asthma symptoms with albuterol and oral corticosteroids.

Citation: Garbutt JM, et al. Home use of albuterol for asthma exacerbations. Ann Allergy Asthma Immunol 2009;102:504-509.

News release issued by the American College of Allergy, Asthma and Immunology (ACAAI)

Health Care Use is Higher in Adult Asthma Patients, Inactivity and Obesity Contributing Factors

ARLINGTON HEIGHTS, Ill., June 10, 2009 - Health care use is higher in adult asthmatic patients when compared with non-asthmatic patients, and inactivity and obesity are contributing to this increase, according to a report published this month in Annals of Allergy, Asthma & Immunology, the scientific journal of the American College of Allergy, Asthma and Immunology (ACAAI).

Shilpa Dogra, MSc, of the Lifespan Health and Performance Laboratory at York University in Toronto, Ontario, Canada, and colleagues, also found that overnight hospital stays were more common in inactive asthmatic patients regardless of body mass index (BMI), whereas both BMI and physical activity were important determinants of physician consultations.

Investigators analyzed self-reported data of an adult population of 6,835 with asthma and 78,051 without asthma from the 2005 Canadian Community Health Survey (CCHS), a nationally representative population-based cross-sectional survey. Their findings include:

Patients with asthma were 2.25 times more likely to have an overnight hospital stay, 1.48 times more likely to have four or more overnight hospital stays, and 2.43 times more likely to have three or more physician consultations compared with patients without asthma.

Inactive patients with asthma were 1.68 times more likely to have an overnight hospital stay and 1.23 times more likely to have three or more physician consultations than active patients with asthma.

Inactive/obese patients with asthma were 2.35 times more likely to have an overnight hospital stay and 2.76 times more likely to have three or more physician consultations than active/ normal weight patients with asthma.

"The most important thing to take from this study is that asthmatics, whether obese or normal weight, can benefit greatly from adopting and maintaining an active lifestyle," said Ms. Dogra. "Health care professionals working with asthmatics should inform their patients of the benefits of an active lifestyle, and the various ways in which they can overcome asthma specific barriers to physical activity, such as exercise-induced asthma. Higher activity levels not only help the individual with asthma, but also have the potential to relieve some of the burden being placed on the healthcare system."