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3/2008

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Wheat grain allergies: an update on wheat allergens

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Key words

Wheat allergens; water/salt-soluble fraction; gluten allergens; ω5-gliadins; wheat isolates.

Summary

Wheat grain is a major staple of our diet. However, proteins derived from wheat grain have been implicated in both respiratory and food allergies, as well as in contact hypersensitivity. Numerous wheat allergens are present in the different fractions of wheat grain: α-amylase/trypsin inhibitor and lipid transfer protein are found in the water/salt soluble fraction, and ω_5-gliadins and LMW-glutenins have been detected in the gluten fraction. This review discusses what is currently known about wheat grain proteins and allergens. The type of IgE-binding profiles (allergens or even epitopes) in patients with wheat food allergy as a function of age, symptoms, or genetic variability of wheat cultivars provides interesting and useful data for developing hypoallergenic foods as well as new tools for diagnostic and therapeutic methods.

Introduction

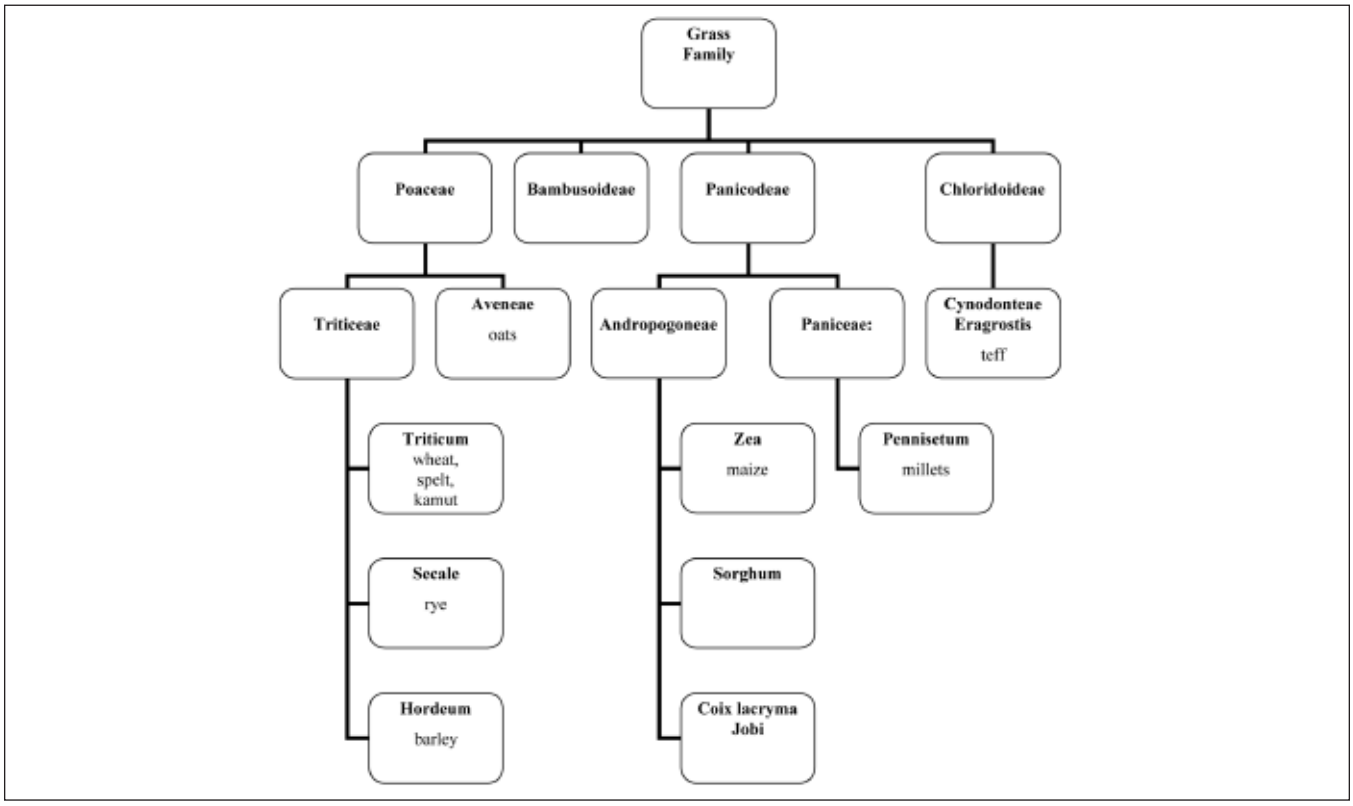
Wheat is the most widely cultivated cereal and represents an important food and dietary protein source worldwide. Wheat proteins can be either ingested or inhaled in the form of raw flour, which can lead to adverse reactions. These adverse reactions cover a broad spectrum of disorders due to different affected pathways and show a variety of clinical manifestations: wheat gluten enteropathy (celiac disease), T-cell-mediated intestinal inflammation, dermatitis herpetiformis, a blistering skin eruption, respiratory allergy to wheat pollen or flour, and wheat grain food allergy. Food allergies include various clinical features, including atopic dermatitis in children, and exercise-induced anaphylaxis, anaphylactic shock, and/or chronic urticaria in adults. This review focuses on what is currently known about the characteristics and structures of wheat grain proteins and wheat grain allergens.

Wheat grain

Cereals are members of the grass family (*Poaceae*) that produce edible dry fruits, known as seeds or kernels. Cereal production is the highest in the world with total annual grain yields estimated in 2006 to be 2020 million tons (mt) (1). Although this includes a large variety of cereal species, three specific cereals together account for >70% of total production: maize (694 mt estimated yield in 2006), rice (631 mt estimated yield in 2006) and wheat (592 mt estimated yield in 2006). Other cereals include barley, sorghum, millets (which consists of a number of small-seeded tropical species), oats and rye, in order of decreasing total production, and finally rare species such as teff (Fig. 1) (2).

The most highly consumed cereals by man include rice and wheat (*Triticum spp.*). Among all the cereal grains, wheat is unique because wheat flour alone has the ability

Figure 1 - Schematic representation of cereal species and grass family classification.



to form a dough that exhibits the rheological properties required for the production of leavened bread and for the wider diversity of foods that have been developed to take advantage of these attributes. Wheat grain is a staple food used to make flour for leavened, flat and steamed breads, cookies, cakes, pasta, noodles and couscous, as well as for fermentation to make beer, alcohol, vodka or biofuel. There are many cultivated species of wheat (Tab. 1), of which two have currently a real economic impact: (A) the common wheat (*Triticum aestivum*) is more typically cultivated in the high latitudes (for example in France, in Canada, in Ukraine). It is largely used to make flour for the bread market; (B) the durum wheat (*Triticum durum*) is cultivated especially in hot and dry zones (southern Europe, for example southern France or Italy). The durum wheat is very rich in gluten, and is used to produce semolina flour and pasta products.

Table 3 - Major cultivated species of wheat

Common Names	Species	Genomes
Bread Wheat	*Triticum aestivum*	Hexaploid
Durum wheat	*Triticum durum*	Tetraploid
Einkorn	*Triticum monococcum*	Diploid
Emmer	*Triticum dicoccum*	Tetraploid
Spelt	*Triticum spelta*	Hexaploid
Kamut® or QK-77	*Triticum plonicum* or *T. durum*	Tetraploid

Wheat species in bold are species most widely cultivated in the world

Wheat grain proteins

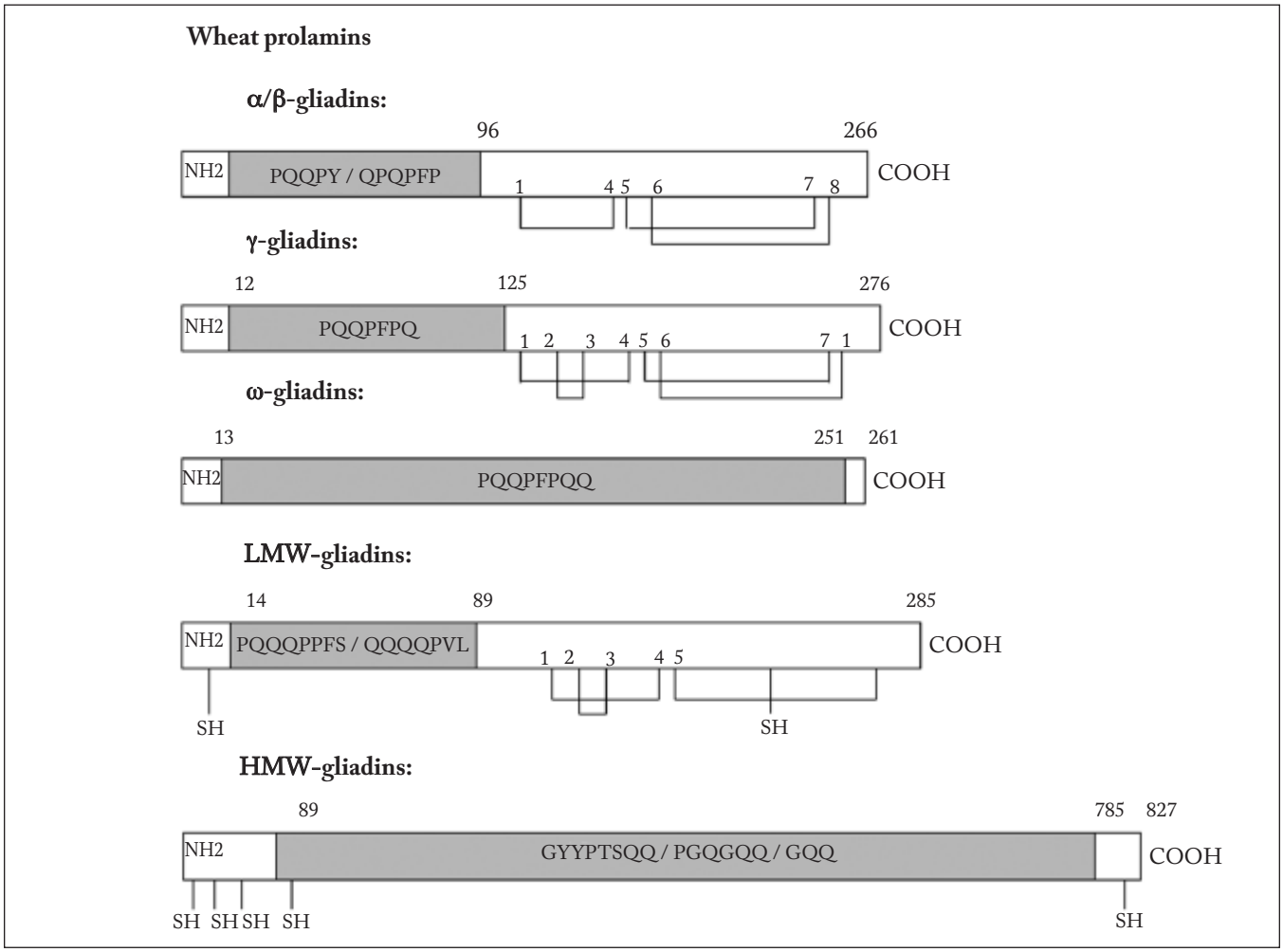
Wheat grains do not contain many proteins (about 10-15% of dry weight) as compared to legume seeds (about 25-30%). Osborne developed a classification of plant proteins based on their solubility in various solvents (3): water (albumins), dilute salt (globulins), aqueous alcohol (gliadins), and dilute alkali or acid (glutenins) solutions.
The water/salt-soluble albumins and globulins are mainly structural proteins and metabolically active enzymes, which contribute about 50% of the total lysine content in

the seed proteins (4). Some proteins have been identified as belonging to the family of α-amylase/trypsin inhibitors and others as part of the lipid transfer family. Several proteins have unknown functions and are not yet well characterized (5, 6). High molecular weight albumins and certain globulins (triticins) are considered to have an additional storage function.

Proteins, between 13 and 18 kDa, corresponding to α-amylase inhibitors and proteins at 37 and 62 kDa corresponding to a peroxidase precursor and a serine carboxypeptidase, respectively, were identified by N-terminal sequencing of the albumin fraction (7). Some authors have also identified several proteins in the globulin fraction (20, 37, 38 and 54 kDa) homologous to barley globulins (2, 8).

The water/salt-insoluble gliadins and glutenins, together known as prolamins or gluten proteins, are the major storage proteins of the wheat grain and are well characterized (9, 10). The name prolamin reflects the fact that these proteins are unusually rich in proline and glutamine (accounting for 30-70% of total amino acids which is typically observed in cereal species) that are found in highly repetitive sequence motifs (2). The main features of prolamins are the high occurrence rate of genetic polymorphisms (one wheat variety can contain more than 50 different prolamins), their similar organization into repetitive and non-repetitive domains and the presence of extensive sequence homologies between the different gliadins and glutenins (Fig. 2). Moreover, wheat pro-

Figure 2 – Schematic structures of some wheat prolamins (according to Shewry et al. 1994 and 2002, Anderson et al. 2001 and Hsia et al. 2001) (2, 102-105). Repetitive domains are shaded and conserved cysteine residues (1 to 8, from α/β-, γ-gliadins and LMW glutenins) forming an intramolecular disulphide bridge are connected by a black line and the corresponding numbers 1-8. SH denotes the positions of cysteine residues in LMW and HMW glutenins not implicated in disulphide bridge



lamins share a great degree of sequence and structural homology with the corresponding proteins in rye and barley (2, 9, 11).

The ethanol-soluble gliadin group comprises over 40 structurally similar monomeric proteins, which account for 30-40% of wheat grain proteins. They are classified as α/β-, γ- and ω-types on the basis of their electrophoretic mobility in acid-polyacrylamide gel electrophoresis (PAGE) (12). The molecular weights (MW) of α/β- and γ-gliadins range from 30-40 kDa and those of ω-gliadins from 40-50 kDa, although their apparent MW in sodium dodecyl sulfate (SDS)-PAGE is much higher (55-75 kDa) (10, 13). Slow- and fast-moving ω-gliadin components are observed in acid-PAGE and were designated by Kasarda et al. (14), as ω_1, ω_2 (slow-moving) and ω_5 (fast-moving) as a function of their mobility and their N-terminal sequences.

The alkali or acid-soluble glutenins account for 35-40% of total proteins and correspond to polymeric proteins linked by intermolecular disulfide bridges (10). They are composed of high molecular weight (HMW) subunits (apparent MW in SDS-PAGE ranges from 80-120 kDa) and of low molecular weight (LMW) subunits. LMW glutenins are further divided into three groups of subunits: the B-group (subunits of 42-51 kDa), the C-group (30-40 kDa), including subunits related to α/β- and γ-gliadins, and the D-group (60-75 kDa) containing ω-gliadin-like subunits.

The availability of complete amino acid sequences of various prolamins has allowed the redefinition of their classification in relation to structural and evolutionary relationships (11). This system of classification assigns all of the prolamins of the Triticeae (wheat, barley and rye) family to three broad groups: sulfur-rich, sulfur-poor and HMW prolamins (Tab. 2) (2). The sulfur-rich prolamins (LMW glutenins, α-gliadins and γ-gliadins) are composed of an N-terminal domain that contains proline and glutamine-rich repeats and a C-terminal non-repetitive domain with even numbers of cysteine residues that form intra-chain disulfide bonds. The non-repetitive domains have been suggested to be rich in α-helices (15). In contrast, ω-gliadins consists almost entirely of repeats and are characterized by a low content of sulfur-containing amino acid residues and a lack of cysteine residues.

Wheat grain allergens

Wheat grain proteins are involved in the three routes of sensitization: inhalation, contact and ingestion (16). Depending on the route of allergen exposure and the underlying immunologic mechanisms, wheat grain allergy can appear as occupational asthma and rhinitis, contact urticaria or classic food allergy affecting the skin, gut, and/or respiratory tract, or as exercise-induced anaphylaxis (17-22).

Baker's asthma is one of the most common forms of occupational asthma. Baker's asthma is an occupational disease affecting 4-10% of bakery workers in European countries (23). Food allergy, defined as adverse immune response to food proteins, affects as many as 6% of young children and 3% to 4% of adults (24). Any protein-containing food may induce an allergic reaction. Wheat is among the six foods, identified by Codex Alimentarius, responsible for approximately 90% of food allergies in children, and in recent years has been increasingly recognized as a cause of food-dependent exercise-induced anaphylaxis (FDEIA) (21). Wheat allergens have been involved in 6% of life-threatening food anaphylaxis between 2002 and 2004 (25) in France, in which exercise could have been an aggravating factor. However, no fatal cases of exercise-induced wheat-related anaphylaxis has been reported to date, despite the frequency of allergy to wheat (25). In Iran, wheat is reported as the most fre-

Table 2 - Classification of wheat grain prolamins (characteristics and groups) adapted from Shewry et al. 2002 (2)

Prolamins (70% of total wheat grain proteins)		
Sulfur-rich (70-80% of prolamins)	Sulfur-poor (10-20% of prolamins)	HMW prolamins (6-10% of prolamins)
α/β-gliadins γ-gliadins B- and C-groups of LMW-GS	ω-gliadins D-group of LMW-GS	HMW glutenin subunits
65-90 kDa	30-45 kDa	30-75 kDa

LMW-GS: LMW glutenin subunits

quent causes of anaphylaxis for children (26). Wheat allergy appears more frequently in Northern (27) than in Southern Europe. In France, wheat ranks as the 8th most frequent food allergen in children and as the 12th in adults (28). It represents 20% of the food allergy clinical pediatric population in the study of Sicherer (29) and 14% in the study of Niggemann (30). In the study of Moneret-Vautrin et al, it represents 10.9% of children and 25% of adults (20). In the cohort of the Isle of Wight on six-year-old children, wheat was the third key allergen giving positive food challenges (31). In contrast, in an American study on food allergy in children, wheat-related food allergy was present in only 2.5% of the cases (32, 33). These numbers could be underestimated because they represent only the most severe cases where hospital care was necessary.

Clinical manifestations of wheat allergy are similar to those of other food allergies, with symptoms at the level of the skin, the gastrointestinal tract and the respiratory tract (17). Clinical patterns in children and adults are different. The main symptoms in children are atopic dermatitis (AD), either alone or associated with respiratory symptoms and digestive problems (27, 34-38). In adults, various clinical features have been identified including FDEIA, anaphylactic shock, angiooedema, irritable bowel syndrome, eosinophilic oesophagitis, rare cases of ulcerative colitis or buccal aphtosis (22, 39-48). Wheat may be introduced very early in the diet, around the fifth month after birth. Moreover, sensitization might occur much earlier through maternal milk, in which the presence of non-degraded gliadins have been reported in breast-feeding mothers not following a specific diet (49, 50). On the contrary, Poole et al. (51), observed that delaying initial exposure to cereal grains until after 6 months may increase the risk of developing wheat allergy. Wheat-dependent exercise-induced anaphylaxis (WDEIA) was mostly observed in adults, and sometimes in children (48, 52, 53). The diagnosis, however, is difficult, as well as the exercise level necessary to induce the symptoms, and the wheat quantities are very variable (53-55).

Water/salt-soluble allergens

Several water/salt soluble proteins have been described as allergens: α-amylase inhibitors, serpin (serine proteinase inhibotor), acyl-coenzyme A oxidase, fructose-biphosphate aldolase and wheat flour peroxidase (56-58). Among them, the α-amylase/trypsin inhibitor family, which include several 12-17 kDa proteins, are considered to play a significant role as allergens for individuals with baker's asthma (56, 59, 60). This protein family is also a relevant sensitizing allergen for wheat food allergic patients (45, 61, 62). A large panel of allergens comprised between 12 and 70 kDa belonging to water/salt soluble proteins have been also described in wheat allergies (16, 63-67). We also have shown that water/salt soluble proteins appeared as significant allergens in 60 children and adult patients with wheat food allergy confirmed by double-blind, placebo-controlled food challenge (DBPCFC) (68). Wheat lipid transfer proteins (LTPs) have been identified as allergens for wheat allergic patients (62, 68, 69). LTP1 (9kDa) and LTP2 (7kDa) were recognized by specific IgE from patients with wheat food allergy; this IgE population also demonstrated *in vitro* cross reactivity to wheat, barley and maize LTPs (69). Recently, a novel cross reactive cereal allergen family, the wheat thioredoxins, has been identified as being related to baker's asthma (70).

Prolamin or gluten (gliadins and glutenins) allergens

All gliadin and glutenin protein fractions have been described as wheat grain allergens in several studies (16, 19, 62, 67, 71-73). Alpha- and ω_5-gliadins are also associated with baker's asthma (74). In a previous study, we observed IgE binding to α/β-, γ- and ω-gliadins in addition to LMW glutenins for wheat allergic children and adults (66). In a more extensive study with 60 patients, we showed that different allergenic profiles could be detected in wheat food allergy , as a function of patient age and symptoms manifested (68). Indeed, α/β- and γ-gliadins (with some proteins of water/salt-soluble fraction) appeared to be more important allergens for children with AD with or without asthma, while ω_5-gliadins were major allergens for adults with WDEIA and/or anaphylaxis (100%) or urticaria (55%). Only 23% of patients with AD and 8% of those with AD and asthma reacted to ω_5-gliadins. B-type LMW glutenin subunits also was identified as significant allergens in adult anaphylaxis cases (62, 68) but also in children (75). In all cases, HMW glutenins were only minor allergens. The specific role of ω_5-gliadins in WDEIA has been demonstrated by several studies (48, 55, 76). Palosuo et al. revealed that ω_5-gliadins are also allergens in children with an immediate-type allergy to wheat or with wheat-induced anaphylaxis (77, 78). The same group showed that ω_5-gliadins induced release of histamine from the basophils of patients with WDEIA but not from those of controls (48). The authors also pre-

Table 3 - Summary of major IgE-binding epitopes identified on prolamin allergens

Proteins	Sequences	Symptoms	References
Gliadins	QQPFP/PQQPF	AD	(84)
γ-gliadins	QQLVPQ/QQSFPQ	WDEIA	(87)
ω_2-gliadins	QQPIPQQ/QQPFPQQ	WDEIA	(87)
ω_5-gliadins	QQXPQQQ/QQSPEQQ	WDEIA, A, U	(85, 87)
LMW glutenins	QQQPP	AD	(82, 83)
HMW glutenins	QQPGQ/QQPGQGQQ/QQSGQGQ	WDEIA	(86)

A: Anaphylaxis; AD: Atopic Dermatitis; U: Urticaria; WDEIA: Wheat dependent-exercise induced anaphylaxis.
X: being F, I, L, Y or S.

sented data hypothesizing that tissue transglutaminase (tTG) in the intestinal mucosa of patients could be activated during exercise. Indeed, tTG-mediated cross-linking of a pepsin-trypsin digested ω_5-gliadin causes a marked increase in binding to IgE (79). Moreover, serum gliadin levels are correlated with clinical symptoms induced by exercise and aspirin in patients with WDEIA (80). The authors suggested that exercise and aspirin facilitate allergen absorption from the gastrointestinal tract. Recently, recombinant forms of ω_5-gliadins demonstrated similar IgE-binding abilities (81).

Several studies have identified IgE-binding epitopes on prolamin allergens and for wheat allergic patients with different symptoms (Tab. 3). A QQQPP motif has been identified as an IgE-binding epitope in LMW glutenin for patients with AD (82, 83). The same group reported IgE-binding abilities of QQPFP and PQQPF motif in gliadin sequence for patients with AD (84). Another study restricted to WDEIA cases described several linear epitopes among ω_5-gliadin repeats (QQXPQQQ with X being I, F, S, Y or L and QQSPEQQ) (85). In this study, LMW glutenins were also detected by IgE in some patients with WDEIA. Several sequences are known for these proteins and some of them contain the motifs QQLPQQQ or QQFPQQQ in their repetitive domains that could cross-react with ω_5-gliadin epitopes. Three IgE-binding epitopes within the primary sequence of HMW glutenin have also been observed for Japanese patients with WDEIA (86). We also reported several IgE-binding linear epitopes on α/β-, γ- and ω-gliadins (ω_2- and ω_5-gliadins) for wheat allergic patients (87). Patients (mainly adults) with anaphylaxis, urticaria of WDEIA, showed strong IgE-binding to sequential epitopes of the repetitive domains of gliadins. Among these repetitive domains, 2 immunodominant epitopes on ω_5-gliadin with a consensus motif of the type QQX_1PX_2QQ (X_1 being L, F, S or I and X_2 Q, E or G) were identified. It is thus interesting to see that IgE of wheat allergy patients of Japanese or French descent recognized the same epitopes. The absence of IgE linear epitopes in children with atopic eczema/dermatitis syndrome leads to the hypothesis that specific responses occur to conformational epitopes.

Gluten modified allergens

The principal properties of the wheat grain reside primarily in the gluten-forming storage proteins of its endosperm. Wheat gluten proteins form a continuous viscoelastic network when flour is mixed with water to form dough (88). While rice is frequently consumed by humans without any processing except cooking, wheat is almost always consumed after processing (89). Bread, pastries, pasta, and noodles are just the few examples of processed products. However, wheat gluten actually exhibits low solubility in aqueous solution (2). This causes limited applications of wheat gluten on various types of food, as solubility is the main characteristic of proteins selected for use in liquid foods and beverages. Furthermore, solubility is closely related to other functional properties of proteins such as foaming, emulsification, and gelling ability (88). This explains why many processes are used to improve solubility of wheat gluten, such as hydrolysis and/or deamidation (89).

These biochemical modifications on gluten proteins are currently being used by various industries (food, cosmetics, pharmaceutical, …). In Japan and several Asian countries, these derivatives or modified wheat products are traditionally used as seasoning. These wheat products could induce a reduction of wheat allergenicity by decreasing the molecular mass of native wheat allergens after enzymatic or acid hydrolysis or deamidation (89-92). On the other hand, these treatments on native proteins could also

uncover allergenic motifs or create neoallergens. Wheat hydrolyzed proteins have been described as being responsible for immediate hypersensitivity reactions such as contact urticaria or anaphylaxis (93-95). Wheat isolate, corresponding to a concentrate of gluten proteins which have been submitted to heat and acid treatments causing a partial deamidation, have been implicated in some cases of allergy restricted to wheat isolates, in the absence of reactivity to unmodified wheat itself (16, 96, 97).

Conclusion

The route of sensitization appears to be important in the development of wheat allergy. Patients with baker's asthma are mostly sensitized to raw flour particles inhaled via the respiratory mucosa, whereas patients with food allergy are primarily sensitized to heat-treated and digested wheat proteins absorbed through the gastrointestinal epithelium. Indeed, Lauriere et al. (*98*), also suggest that state and route of exposure to very similar structures probably orientate the pattern of epitope reactivity and clinical manifestations. Nevertheless, review of the literature indicates that a large variety of wheat grain proteins have been identified as allergens in all pathologies studied. The type of IgE-binding profile in patients with wheat food allergy is correlated to age and symptoms that manifest. The elucidation of the major IgE-binding epitopes on wheat allergens could provide a useful tool for developing hypoallergenic foods as well as new diagnostic techniques and immunotherapy for patients with wheat grain allergies. Monitoring of the development of wheat allergies in patients is critical in determining relationships between wheat protein epitopes and allergy persistence. Indeed, this approach has already proven useful for egg and milk allergies (99, 100). Furthermore, studies on genetic variability of wheat cultivars represent a new and interesting approach for developing ways of decreasing allergenicity of wheat products (101).

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Characteristics of patients with allergic polysensitization: the polismail study

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Key words

Rhinitis, asthma, polysensitization, quality of life

Summary

Background: *The natural history of respiratory allergy is commonly characterized by a worsening of symptom severity, frequent comorbidity of rhinitis and asthma, and polysensitization to aeroallergens. The polysensitization phenomenon starts since childhood and is rare to find monosensitized adult patients. However, there are few studies investigating the characteristics of polysensitized patients.* ***Methods:*** *This study was performed on a large cohort of patients with allergic rhinitis (assessed by ARIA criteria) and/or mild to moderate asthma (assessed by GINA). The kind and the number of sensitizations, their patterns, and the relation with quality of life (QoL) measured by the Juniper's RQLQ questionnaire, were evaluated.* ***Results:*** *Globally 418 patients*

(50.2% males, 49.8% females, mean age 26.4 years, range 3.5-65 years, 64 smokers, 371 non-smokers) were enrolled: 220 had allergic rhinitis alone, and 198 allergic rhinitis and asthma. The mean number of sensitizations was 2.6. Three hundred-five patients (73%) had persistent rhinitis (PER), 220 of them with moderate-severe form. There was no significant difference in rate of rhinitis and asthma in monosensitized or polysensitized patients. Most patients were sensitized to pollens, whereas only 24.2% of them were sensitized to perennial allergens. Polysensitization was significantly associated with some issues of QoL, confirming previous findings, but not with number of sensitizations. ***Conclusions:*** *This study provides data confirming for polysensitized patients the relevance of ARIA classification of AR. PER is the most common form of AR in this cohort, symptoms are frequently moderate-severe, and asthma is present in about the half of patients with AR.*

Introduction

Allergic rhinitis (AR) is very frequent as it affects up to 40% of people (1) and its prevalence is still increasing (2). Social and economic costs are substantial because of such high prevalence and daily activities, productivity, and quality of sleep are significantly affected (3). AR classification has been recently revised by the Allergic Rhinitis and its Impact on Asthma (ARIA) group (1). This classification includes a measurement of the frequency and duration of the symptoms. Thus, intermittent AR (IAR) is defined by symptoms occurring for <4 days/week or <4 consecutive weeks. Persistent AR (PER) is defined by symptoms occurring for >4 days/week and >4 consecutive weeks. Additionally, a severity scale of mild to moderate-severe was included in the revised classification. This new classification has been object of some studies that compared it with the old one that was based on the period around the year of symptom occurrence, such as seasonal or perennial.

Demoly et al. assessed the characteristics of patients presenting for AR during the spring season (SAR) and patients presenting during the fall-winter season (PAR) (4). Their results show that SAR was not synonymous of IAR as well as PAR is not equivalent to PER. More recently, Bauchau and Durham performed a similar study in a larger population (5). They concluded that the classic types of SAR/PAR cannot be used interchangeably with the new classification of IAR/PER, as they do not represent the same status of disease. In addition, they stated that PER constitutes a distinct disease.

Moreover, it is well known that AR is frequently associated with asthma as evidenced by several studies (6, 7). Asthma is classified on the basis of severity and duration of symptoms as intermittent or persistent that may include mild, moderate or severe type (8). In addition, AR is characterized by the phenomenon of polysensitization, i.e. allergic patients tend to become sensitized to more allergens over the time (9).

Quality of life (QoL) is impaired in patients with AR (10) and asthma (11). This issue has been assessed in a number of studies, but the global relationship among type of AR, association with type of asthma, number of sensitizations, and QoL has been not investigated still now.

Therefore, this study was aimed at evaluating these parameters in a large cohort of Italian patients suffering from AR.

Material and methods

Study Design

The study was conducted in 26 Allergy Centres homogeneously distributed in Italy. It was designed to include samples representative of the general population and to have the ability to identify the new diagnosed cases. The study was approved by the Review Board of each participating center and an informed consent was obtained from each patient. The first part of the study was performed during autumn-winter 2005.

Subjects

418 patients (50.2% males, 49.8% females, mean age 26.4 years, range 3.5-65 years, 64 smokers, 371 non-smokers) with allergic rhinitis were prospectively and consecutively

evaluated. A detailed clinical history was taken and a complete physical examination was performed. The patients were included in the study on the basis of a clinical history of allergic rhinitis and presence of nasal symptoms according to validated criteria (1).
The diagnosis of intermittent or persistent allergic rhinitis was made on the basis of a history of nasal symptoms and positive skin prick test (1).
Skin prick tests were performed as stated by the European Academy of Allergy and Clinical Immunology (12). The panel consisted of: house dust mites (*Dermatophagoides farinae* and *pteronyssinus*), cat, dog, grasses, *Compositae*, *Parietaria officinalis*, birch, hazel, olive tree, cypress, *Alternaria*, *Cladosporium*, *Aspergillus* (Stallergenes, Milan, Italy).
The diagnosis and severity classification of asthma were made according to GINA criteria (8). Moreover, patients gave a subjective judgment of the severity of their condition and the use of drugs in the past 12 months by a visual analogue scale (VAS).
Quality of Life was evaluated by the Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ); it consists of 28 items distributed in seven dimensions: sleep problems (3 items), non-hay fever symptoms (7 items), practical problems (3 items), nasal problems (4 items), eye symptoms (4 items), activities (3 items), and emotions (4 items). Responses to the items are scored on a 7-point Likert scale, while dimensions and overall scores are scored on a 0-6 scale, the lower the score, the better the HRQL (10).

Statistical Analysis

Continuous and/or discrete parameters were reported as mean, SD, third quartile, and frequency. Categorical parameters were reported in contingency tables.
Homogeneity of data was evaluated by Fisher exact test. The significance of the values concerning the relationship coefficient was calculated by Student's t test. The analysis on factors determinant the polysensitization was performed by polycotomic logistic regression, considering as dependent variable: gender, age, severity of rhinitis, VAS values, and QoL. About these parameters, coefficient values, standard error, coefficient/standard error ratio, and Odds ratio were reported.
The p value concerning the statistical significance was set at 0.05. Statistical analysis was performed by statistical package BMDP Dynamic produced by BMDP Statistical software, Inc.

Results

The distribution of patients on the basis of their age shows that 55 (12.6%) had < 10 years, 113 (25.7%) 11-20 years, 112 (25.7%) 21-30 years, 101 (23.2%) 31-40 years, 37 (8.5%) 41-50 years, and 18 (4.1%) > 50 years. Of the 418 patients included in the study, 220 had AR alone, 198 (47.4%) had AR associated with asthma, as reported in table 1. One hundred-thirteen patients had IAR, corresponding to 29% of sample. Three hundred-five patients had PER (73%). Mild severity was present in 34 patients with IAR and 85 with PER. Moderate-severe form was in 79 patients with IAR and 220 with PER. Thus PER was the type more frequent as well as moderate-severe was the severity more relevant. The type and severity of AR did not affect the association with asthma (Fisher test = 0.7476, Prob. = 0.8620).
Concerning asthma severity, 102 patients had intermittent form, and 96 persistent form (46 mild, 44 moderate, and 6 severe). However, the type and severity of AR did

Table 1 – Type and severity of allergic rhinitis and asthma in 419 patients with allergic rhinitis

Rhinitis Classification	Rhinitis alone	Rhinitis and Asthma	Total
Mild Intermittent	7.3% (16/220)	9.1% (18/198)	8.1% (34/418)
Moderate-Severe Intermittent	20% (44/220)	17.7% (35/198)	18.9% (79/418)
Mild Persistent	20% (44/220)	20.7% (41/198)	20.3% (85/418)
Moderate-Severe Persistent	52.7% (116/220)	52.5% (104/198)	52.6% (220/418)
Asthma Classification			
Intermittent	51.5% (102/198)		
Mild Persistent	23.2% (46/198)		
Moderate Persistent	22.2% (44/198)		
Severe Persistent	3% (6/198)		

not affect the severity of associated asthma (Fisher test = 2.6320, Prob. = 0.4519).
Only 10% of patients were monosensitized, the number of sensitizations is reported in figure 1. The number of patients was progressively decreasing with the increase of number of sensitization, but it was not associated with possible presence of asthma (Fisher test = 0.6843, Prob. = 0.7102).

Figure 1 - Rhinitis and rhinitis plus asthma according to number of sensitizations

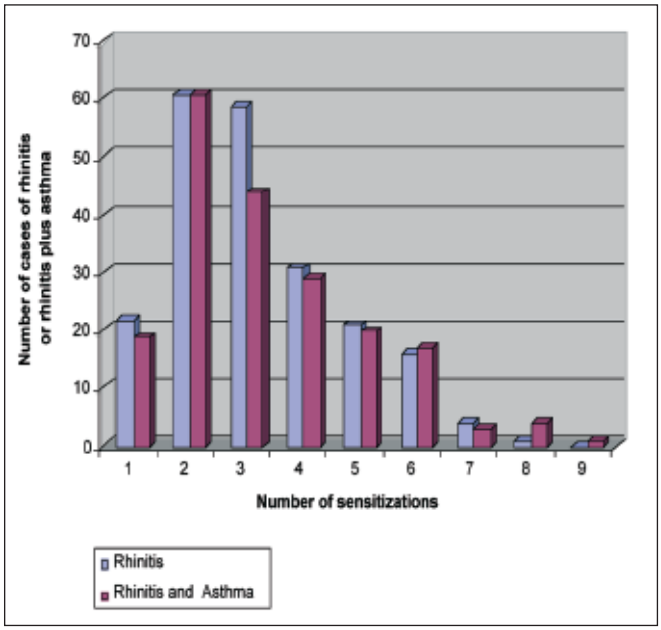


Figure 2 - Severity of rhinitis and kind of sensitization

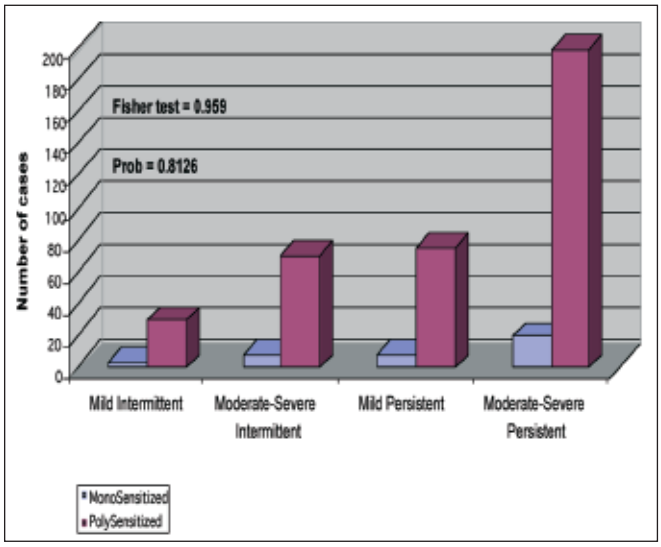
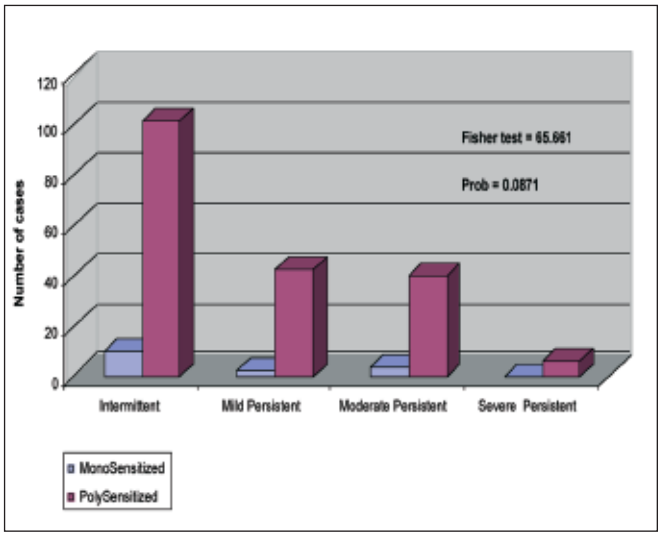


Figure 3 - Severity of asthma and kind of sensitization



Globally considering mono- or polysensitization as reported in figures 2 and 3, polysensitization did not affect the type and severity of AR (Fisher test = 0.9529, Prob. = 0.8126), whereas there was a trend for the association between polysensitization and asthma severity (Fisher test = 6.5661, Prob. = 0.0871), indeed only polysensitized patients had severe persistent asthma.
About specific sensitizations, grasses were the most relevant allergen, followed by house dust mites and several pollens, as reported in table 2. About the symptoms severity assessed by VAS, ocular symptoms had a mean value of 5.5 (S.D. 2.4), nasal symptoms 7.3 (S.D. 2.1), and bronchial symptoms 3.5 (S.D. 3.1).
Concerning the use of drugs assessed by VAS, oral antihistamines were the most consumed (5.9, S.D. 3.1), followed by topical nasal corticosteroids (4.0, S.D. 3.3), in-

Table 2 - Type of allergen sensitizations to SPT

Allergen	Number of positive SPT	%
Grasses	295	76.4
Dermatophagoides	183	47.4
Parietaria	150	38.9
Betulaceae	148	38.3
Olive	103	26.7
Ragweed	90	23.3
Cat	87	22.5
Dog	51	13.2
Alternaria	40	10.4
Cypress	37	9.6

Table 3 - Relationship among severity and diagnosis of rhinitis and asthma, symptoms score assessed by VAS, drugs use assessed by VAS, age, and sensitizations

Relationship coefficient	Severity	Prob.	Signif.
Age	0.0498	0.3672	N.S.
Diagnosis	-0.0233	0.6732	N.S.
Sensitizations	-0.0399	0.4701	N.S.
Symptoms by VAS			
Ocular	0.0184	0.7391	N.S.
Nasal	0.2335	0.0000	P<0.01
Bronchial	-0.0925	0.2143	N.S.
Drugs use by VAS			
Inhaled corticosteroids	0.0899	0.2032	N.S.
Beta2 long acting	0.0312	0.6594	N.S.
Beta2 short acting	0.1827	0.0093	P<0.01
Nasal corticosteroids	-0.0407	0.5652	N.S.
Antihistamines	-0.0123	0.8621	N.S.
Antileukotrienes	0.2201	0.0016	P<0.01

Table 4 - Polycotomic logistic regression model for relating QoL issue with polysensitization

						C.I. 95%	
Factor	Coefficient	E.S.	Coeff./E.S	Prob.	Odds ratio	Lower Limit	Higher Limit
Activity 1	0.208	0.075	2.79	P<0.01	1.2	1.1	1.4
Bad Sleep	0.245	0.098	2.5	P<0.05	1.3	1.1	1.5
Headache	0.164	0.065	2.52	P<0.05	1.2	1	1.3
Eye complaint	0.126	0.064	1.96	P=0.05	1.1	1	1.3
Irritability	0.168	0.072	2.33	P<0.05	1.2	1	1.4

haled corticosteroids (2.6, S.D. 3.4), long-acting Beta2 agonists (2.0, S.D. 3.1), short-acting Beta2 agonists (1.4, S.D. 2.3), and antileukotrienes (1.2, S.D. 2.8).

A significant relationship was found between severity of rhinitis and nasal symptoms score by VAS, moreover asthma severity was related with short acting Beta2 agonists use and antileukotrienes use, as reported in table 3.

However, the polycotomic logistic regression, considering as dependent variable the sensitizations number, does not single out significant prognostic factors.

As to QoL assessment by the specific questionnaire, the most impaired item was activities, followed by practical problems and nasal symptoms. A significant relationship between polysensitization and some issues of QoL was found, as reported in table 4.

Discussion

In this study, we addressed several questions about the characteristics of patients with AR assessing the new ARIA classification of AR (1) and the relationships with asthma co-morbidity and number of sensitisations.

Polysensitisation is an immunological phenomenon that is clinically relevant and seems to be increasing from an epidemiological point of view as recently reported in Italian surveys (7, 13, 14). Therefore, the increasing number of sensitisations seems to characterize the natural history of allergic patient and may represent an evolutionary aspect of allergic reaction. The problem concerns whether this phenomenon may cause an impairment of clinical picture. This study provides some informations about this issue.

Firstly, most patients (73%) have the PER form and the most frequent severity grade is moderate-severe (71.5%). This finding is relevant and appears to be different in comparison with previous studies conducted in general adult population: in Bauchau's study the percentage of patients with PER was 29%. This contrasting finding may be partially explained by the relevant number of sensitizations in our cohort and may depend on the type of studied populations. Indeed, two recent studies conducted on selected patients showed that the percentage distributions are inverse in comparison with Bauchau's study (13, 15). Moreover, the pollen seasons are very prolonged in Italy in comparison with other European countries, mainly concerning Northern ones. This fact may account the persistence of symptoms in our patients.

Secondly, about half patients with AR have also asthma. Thus, asthma represents an important co-morbidity for AR. This finding confirms the statements of ARIA document (1). About asthma severity, most patients had the intermittent form, whereas almost all patients with persistent asthma had mild-moderate severity of symptoms. This finding is partially conflicting with other surveys on asthma. Probably, it might be explained by the concomitant AR. Indeed, it is well known that AR represents a worsening factor for asthma (2).

Thirdly, most patients (90%) are polysensitized. This finding is not surprising as it was reported in previous studies (9,11). This finding outlines the clinical relevance of polysensitization as this phenomenon is very frequent and may influence the aptitude of physicians in managing allergic patients, mainly concerning the prescription of specific immunotherapy.

Fourthly, polysensitization does not appear to be relevant for asthma co-morbidity, and severity of both rhinitis and asthma. Also this finding is partially conflicting with a previous survey conducted in young AR patients (16), even though it seems singling out a trend for asthma co-morbidity and severity of both rhinitis and asthma in patients with polysensitisations. Probably these conflicting findings might depend on several confounding factor: age of patients, type of sensitisations, and overall duration of rhintis. In this regard, a very recent paper showed that the duration of allergic rhinitis and mite sensitisation are relevant risk factor for inducing spirometric impairment (17). Moreover, drug therapy partially could interfere the nose-bronchi relationship, even though it is well known that pharmacotherapy is not able of modifying the natural course of allergy.

Grasses are the most relevant allergen followed by house dust mites. This finding confirms previous studies conducted in Italy (16).

In addition, there is a significant relationship between degree of symptoms and severity of rhinitis as well as between use of both short acting bronchodilators and antileukotrienes and asthma severity. It is to consider that the studied population was essentially composed by patients with AR. However, this study was conducted during a season, i.e. autumn-winter, characterized by reduced severity of symptoms. This issue may constitute a limitation of this study.

Finally, polysensitisation was significantly associated with some issues of QoL, confirming previous findings (18). Therefore, polysenstization may represent an aggravating factor that contributes to impair clinical features in allergic patients.

In conclusion, this study provides new data confirming the relevance of ARIA classification of AR in polysensitized patients. PER is the most common form of AR in this cohort, symptoms are frequently moderate-severe, and asthma is present in about half patients with AR. Therefore, the new ARIA classification and its recommendations should be firmly considered mainly in patients with polysenstizations. Thus, polysenstitization has to be considered as a relevant aspect in allergic patients and has to be carefully evaluated, mainly if immunotherapy has to be prescribed. However, it is needed to address further studies to such issue to confirm these findings.

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Effect of statins on fibroblasts from human nasal polyps and turbinates

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Key words

Airway remodelling, fibroblasts, nasal polyp, statins, turbinate

Summary

Background: *Statins are serum cholesterol-lowering agents used for the prevention and treatment of atherosclerotic vascular disease. There is, however, growing evidence that statins have immunomodulatory and anti-inflammatory activities and may prove invaluable in the treatment of immunological and inflammatory disorders.* ***Objective:*** *On these basis we evaluated the effect of statins on the proliferation of fibroblasts derived from human nasal polyps and turbinates and determined their ability to modulate airway remodelling.* ***Methods:*** *Fluvastatin (0.01-0.1-1 μM), Atorvastatin (0.1-1-10 μM) and Simvastatin (0.1-1-10 μM) were tested on cultured fibroblasts derived from human nasal polyps and turbinates stimulated or not with Fibroblast Growth Factor β (10 ng/ml). All cultures were treated with ^{3}H-Thymidine (1 μCi/ml) to test cell proliferation.* ***Results:*** *Our results show that proliferation of turbinate-derived fibroblasts is significantly inhibited by the three statins. Fluvastatin is already effective at the lowest dose (0.01 μM), whereas Atorvastatin and Simvastatin act at the plasmatic peak concentration (1 μM). No significant effect was found on fibroblasts derived from nasal polyps, except for Simvastatin which was effective after 144 hours of stimulation. Conclusions: These drugs show a remarkable antiproliferative effect and their different outcome depending on the different kind of fibroblasts* in vitro *is prompting news in the studies about statin use for the treatment of chronic inflammatory diseases.*

* Authors contributed equally to this work

Abbreviations: IFNγ, interferon-gamma; MHC, Major Histocompatibility Complex; FBS, Fetal Bovine Serum; FGF, Fibroblast Growth Factor; DMSO, dimethyl sulfoxide; SE, standard error; COPD, Chronic Obstructive Pulmonary Disease

Introduction

Statins, the 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors, are effective serum cholesterol-lowering agents used in clinical practice for the prevention and the treatment of atherosclerotic cardiovascular diseases (1).

However, the observed clinical benefit with statin therapy is much greater than expected through the reduction of cholesterol levels alone (2). Clinical studies and *in vitro* experiments show, in fact, that these drugs may have beneficial effects in a broad range of inflammatory conditions.

Besides, there is growing evidence that statins have the potential to modify T lymphocyte-driven disease through the ability to inhibit the interaction among adhesion molecules (3), reduce cytokine expression, mobilize endothelial progenitor cells, interact beneficially with the renin-

angiotensin system (4-7) and decrease IFNγ-induced expression of MHC-II on Antigen Presenting Cells (8, 9).
The mechanisms responsible for these anti-inflammatory effects are currently still widely unknown.
The nasal mucosa is the first line of defense against pathogenic and non-pathogenic antigens in the air: normal breathing is through the nose and most particles are filtered there (10). The nasal mucosa has more or less the same histology as the bronchial mucosa except for the muscle layer which is present only in the bronchi. If there are any defects in these mechanical barriers, the respiratory mucosa can be injured and subsequently an inflammatory reaction will follow (11). During the latter the inflammatory cells recruited into the airways release products able to damage the surface epithelium and the underlying interstitial tissues. They also stimulate fibroblast proliferation and the extracellular matrix component deposition below the epithelial basement membrane (12).
These alterations, which include also transformation of fibroblasts into myofibroblasts, are commonly known as part of airway remodelling (13). Consequently, any treatment directed to suppress these inflammatory responses may have therapeutic benefits.
In some studies statins were able to reduce α-SMA expression and *in vitro* proliferation of fibroblasts derived from different organs, including the lower airways (14-18). On the other hand, a recent clinical report demonstrated that statins may induce nasal polyposis (19).
Considering that little is known about statin effects on upper airways, the aim of our study was to determine whether Fluvastatin, Simvastatin or Atorvastatin might inhibit proliferation of fibroblasts derived from human healthy turbinates, assumed as control fibroblasts, and nasal polyps, assumed as triggered cells.

Methods

Preparation and proliferation of nasal primary fibroblasts

Fibroblasts were obtained from nasal polyps of six patients and from turbinates of seven healthy subjects. None of the patients received anti-histamines and anti-inflammatory drug treatment (including nasal steroids) during a minimum of 2 weeks before cells were isolated. No difference in age, allergy and tobacco smoke was recorded in the two groups. None of them was on immunotherapy.
Tissues derived from nasal polyps and turbinates were cut into small fragments and incubated in RPMI-1640 (Euroclone, Milan, Italy) medium containing a mixture of 10 UI/ml DNAse, 500 UI/ml collagenase type IV and 30 UI/ml hyaluronidase (all enzymes purchased from Sigma-Aldrich, Milan, Italy) on a magnetic stirrer for 2 hours at 37°C. The cells were then cultured in RPMI supplemented with 10% FBS, glutamine and antibiotics (Euroclone, Milan, Italy) for at least 24 hours. The fibroblast cultures were characterized by flow cytometry using the specific antibody CD90 (Instrumentation Laboratory, Milan, Italy). All cultures used in the study were >95% $CD90^+$.
Fibroblasts were plated in 96-well microtitre plates at a density of approximately $5x10^3$ cells/well in 0.2 ml of RPMI plus 10% FBS and allowed to attach. After 24 hours of incubation the medium was replaced by 0.2 ml RPMI FBS-free. The day after the medium was replaced with RPMI 2% FBS and different concentrations of Fluvastatin (0.01-0.1-1 μM), Simvastatin (0.1-1-10 μM) or Atorvastatin (0.1-1-10 μM) were added. FGF (10 ng/ml) was used as positive control. Statins were tested both alone and in combination with FGF for 48, 96 and 144 hours. Cell viability was assessed by trypan blue dye exclusion and resulted >85% in all experiments. ^{3}[H]-thymidine was added during the last 18 h. The medium was then removed, the cells harvested and thymidine incorporation was measured by a beta-counter and expressed in Count per minute (Cpm). All experiments were performed in triplicate.

Statins

Fluvastatin, Simvastatin (used in the active form as sodium salt) and Atorvastatin were dissolved in DMSO to a high concentration stock solution, then further diluted in DMSO to obtain a 1000-fold higher range of concentrations than the final working one which contains 0.1% DMSO. In every set of experiments fibroblasts from three wells were treated with 0.1% DMSO alone in order to evaluate its effects on cells.

Statistical Analysis

Data are expressed as mean ± SE. Data were analyzed by non-parametric Wilcoxon test. Data were considered significant when the p value was <0.05.

Results

We investigated the efficacy of different statins (Fluvas-

tatin, Simvastatin and Atorvastatin) on fibroblast primary culture.
For this purpose we tested proliferation of turbinate and nasal polyp fibroblasts using different incubation-times (48, 96, 144 hours).
In all experiments the FGF-induced proliferation was significantly increased, compared to non-stimulated cell cultures ($p < 0.05$). Proliferation of cells stimulated with 0.1% DMSO did not statistically differ from non-stimulated cell cultures.
In preliminary experiments the most effective concentration was 0.1 μM for Fluvastatin and 1 μM for Simvastatin and Atorvastatin (data not shown) and consequently these were the concentrations of drugs used in all following set of experiments.

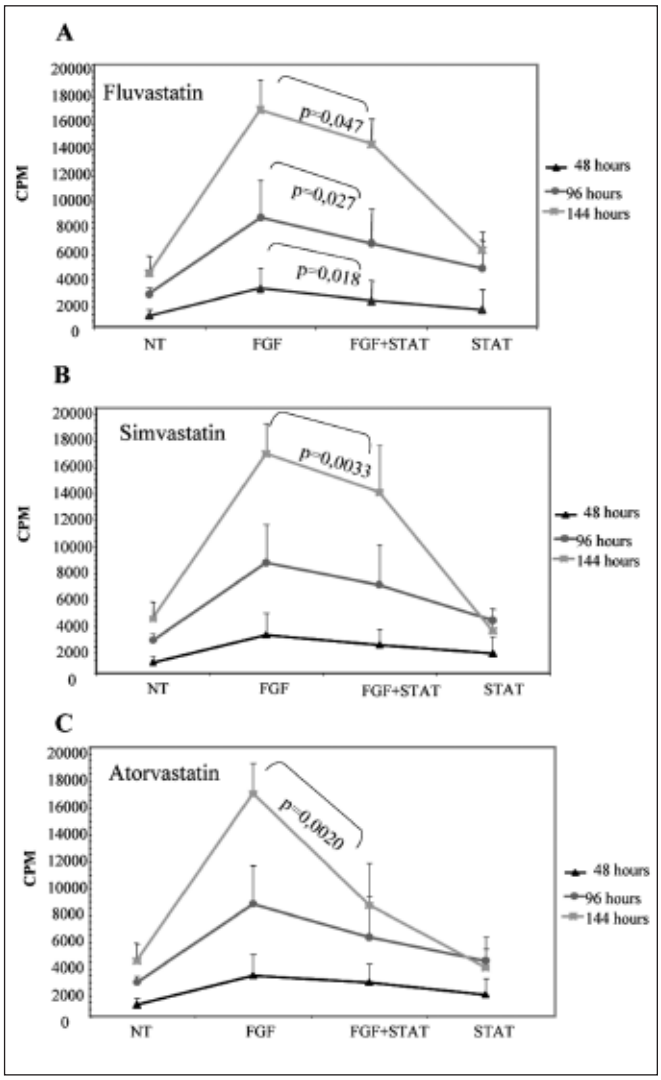
Fibroblasts from turbinates

Fluvastatin 0.1 μM significantly inhibited FGF-induced proliferation of fibroblasts derived from turbinates, when tested at 48 (p=0.018), 96 (p=0.027) and 144 hours (p=0.047).
Simvastatin and Atorvastatin 1 μM were significantly effective on turbinate fibroblast cultures carried on for 144 hours (p=0.0033 and p=0.0020 respectively) (Fig. 1).

Fibroblast from nasal polyps

Statins did not show any response on nasal polyp fibroblast proliferation. Interestingly Simvastatin 1 μM at 144 hours, however, had a significant effect (p=0.0018) (Fig. 2).
We compared the response of turbinate and nasal polyp fibroblasts to each statin to verify the effects of these drugs both in normal conditions and in chronic inflammation.
Our results demonstrated that Fluvastatin and Atorvastatin had a higher anti-proliferative effect on turbinates than on nasal polyps (Tab. 1).
It is, however, interesting to evidence the effect of Simvastatin on nasal polyps too. This drug, in fact, shows a more remarkable anti-proliferative effect on turbinates and no effect on nasal polyps, except for 144 hours incubation-time (Tab.1).

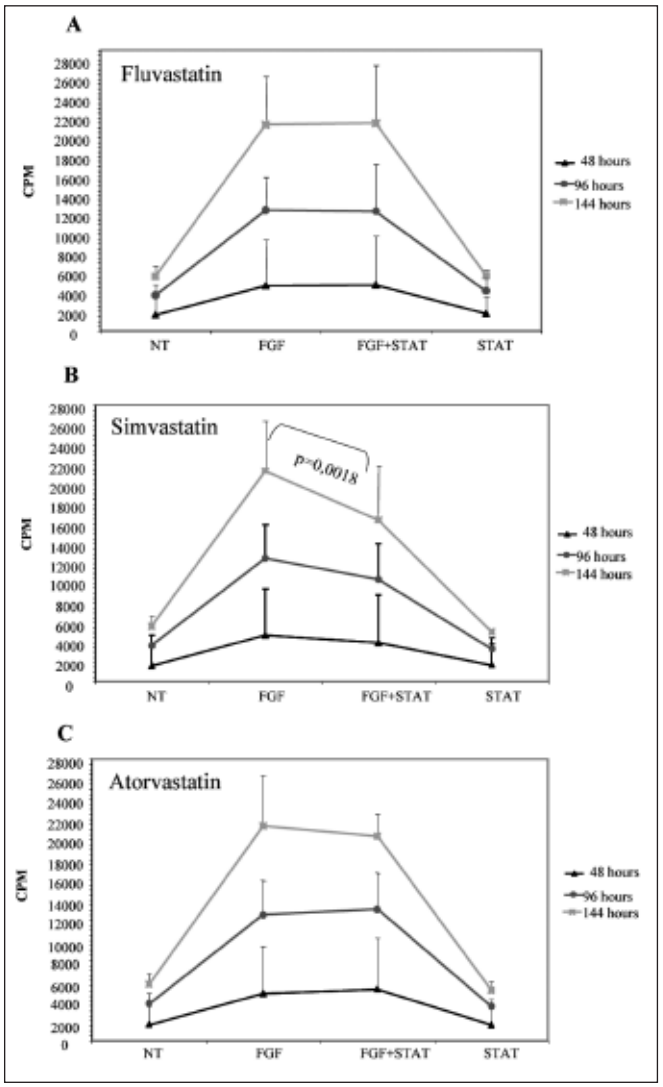
Figure 1 - Effect of Fluvastatin,Simvastatin and Atorvastatin on proliferation of turbinate fibroblasts. A) Fluvastatin; B) Simvastatin; C) Atorvastatin. Data are expressed as mean ± SD. Wilcoxon test was used for statistical analysis and data were considered significant when the p value was <0.05



Discussion

Statins are widely prescribed in cardiovascular disease patients and in view of their pleiotropic anti-inflammatory properties, not related to their cholesterol lowering activity (2), a possible use in other chronic inflammatory diseases may be envisaged. Nowadays quite a global view of some chronic disorders, such as atherosclerosis, suggests that a more general approach also for treatment should be considered. Actually, Simvastatin was shown capable of downregulating human lung fibroblast proliferation and differentiation, α-actin expression and α-actin mRNA expression, in pulmonary idiopathic fibrosis (17, 20). If such an effect is also detectable in asthma,

Figure 2 – Effect of Fluvastatin,Simvastatin and Atorvastatin on proliferation of nasal polyp fibroblasts. A) Fluvastatin; B) Simvastatin; C) Atorvastatin. Data are expressed as mean ± SD. Wilcoxon test was used for statistical analysis and data were considered significant when the *p* value was <0.05



it might be conceivable to think about if, when and how to use these drugs in asthma patients, although the very recent report by Menzies et al. (21) suggests no anti-inflammatory activity of Simvastatin in such patients. Another lung disease which might become a potential target of statins is COPD, this also considering the age of COPD patients and the frequency of cardiovascular comorbidities.

Surprisingly, a recent clinical well-documented report (19) clearly highlighted the relationship between statin assumption and nasal polyposis appearance. This could be seen as a contra-indication in using statins in subjects susceptible to develop nasal polyposis, but at present there is still no method to predict susceptibility to nasal polyposis. Because of this conflicting issue, we designed the present study to investigate statin potential effects on fibroblasts, one of the structural components of nasal polyps.

After one week of culture, an inhibitory effect of statins was consistent through all the experiments performed with turbinate fibroblasts, as far as the spontaneous and induced proliferation is concerned. However, Fluvastatin already showed an earlier inhibitory effect at 48 hours. These experimental data are of interest since a one-week culture is more similar to the long statin-cell interaction *in vivo*. Shorter culture experiments were performed to evaluate the possible fast effect of statins, which was just the case of Fluvastatin.

No effect on nasal polyp fibroblasts has been detected, except for the effect of Simvastatin after a one-week culture. Our experimental evidences suggest a class related effect of statins in controlling turbinate fibroblast proliferation, which is not the case of the same type of cells derived by nasal polyps. This might imply statins are effective in controlling proliferation of "normal" nasal fibroblasts (the ones derived from turbinates), but they are almost ineffective on nasal polyp derived fibroblasts. Moreover, their pleiotropic effects, already observed in previous studies (2, 3, 8, 22), demonstrate that the use of one type of statin may induce different results with respect to another compound of the same class.

Our report reveals the different behaviour of fibroblasts depending on their origin and is the first to investigate different statins and their effects on upper airways fibroblasts. Bearing in mind the report by Bucca and coworkers (19), the question was: are statins inducing nasal polyposis or have they no control on cell proliferation? On the basis of our study, since fibroblasts are a component of polyps, we may exclude the first possibility. In addition, we do not have evidence of an inhibitory effect on nasal polyp fibroblast proliferation, except for a marginal one exerted by Simvastatin.

We know fibroblasts are just one of the cell components of nasal polyps but in light of the published literature concerning statins and fibroblasts in lower airways, we considered it interesting to investigate these cells in our experimental model, although being aware of the fact that results obtained in animal models and *in vitro* may differ from those obtained in human beings.

The conclusions on the basis of the herein reported data

Table 1 - Resumptive scheme of Fluvastatine, Simvastatine and Atorvastatine effect on nasal polyp and turbinate fibroblasts

	FGF/FGF+Statin	Inhibition of proliferation	
Fluvastatin			
48 hours	CPM	%	↑
Nasal polyps	4633,94/4712,99	-1,7%	↓
Turbinates	2888,92/1949,16	+32,5%	
96 hours	CPM	%	
Nasal polyps	7825,83/7641,46	+2,3%	↓
Turbinates	8327,5/6342,5	+23,8%	↓
144 hours	CPM	%	
Nasal polyps	8841,66/9081,66	-2,7%	↑
Turbinates	16514/13894,16	+15,8%	↓
Simvastatin			
48 hours	CPM	%	
Nasal polyps	4633,94/4712,99	+16,0%	↓
Turbinates	2788,92/2142,92	+25,8%	↓
96 hours	CPM	%	
Nasal polyps	7825,83/7641,46	+17,4%	↓
Turbinates	8327,5/6649,55	+20,1%	↓
144 hours	CPM	%	
Nasal polyps	8841,66/6012,75	+31,9%	↓
Turbinates	16514,75/13584,58	+17,7%	↓
Atorvastatin			
48 hours	CPM	%	
Nasal polyps	4667,36/5123,09	-9,7%	↑
Turbinates	2981,78/2440,73	+18,1%	↓
96 hours	CPM	%	
Nasal polyps	7825,83/7930,96	-1,3%	↑
Turbinates	8327,5/6649,55	+29,4%	↓
144 hours	CPM	%	
Nasal polyps	8841,66/7272,16	+17,7%	↓
Turbinates	16514,75/13584,58	+50,1%	↓

CPM= Count per Minute

are: a) other mechanisms, besides fibroblast proliferation, are involved in nasal polyps induced by statins; b) statins are effective drugs in inhibiting resting or normal upper airway fibroblasts. This may not be the case of fibroblasts chronically triggered by inflammatory events, as in nasal polyposis; c) the use of statins in asthmatic patients, needing statins for a comorbidity, may not be contra-indicated but it may be even favourable if the positive effect seen in pulmonary idiophatic fibrosis is valid in asthma too. These drugs, in fact, might exert an effect on airway remodelling, namely on fibroblast to myofibroblasts differentiation. More studies should be designed to explore mechanisms of action, differences among statins and statins role in COPD.

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CD34+ Hemopoietic Precursor and Stem Cells traffic in peripheral blood of Celiac Patients is significantly increased but not directly related to epithelial damage severity

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Key words

CD34+ cell, Celiac Disease, Chronic Inflammatory Disease, CXCR3, CXCR4, Hemopoietic Precursor Cell, iNKT, NFkappaB, Niches, NK cell, Refractory Celiac Disease, Stem cell, T cell, Vbeta8.2

Summary

Celiac disease (CD) is a chronic inflammatory enteropathy of the small bowel resulting from a local TH1-mediated reaction to wheat gliadins and barley, rye and oat prolamins with the development of auto-antibodies to transglutaminases. As well as for other chronic inflammatory diseases, genetic background and environmental factors participate to pathogenesis. An increased traffic of CD34+ hemopoietic precursor and stem cells (HPC) has been reported in peripheral blood (PB) of subjects with allergic diseases that share in their pathogenesis immuno-mediated reactions, genetic and environmental factors. The aim of the present work was to investigate the CD34+ cell traffic and H2 / H1 polarization of lymphoid T-cell lineage, in the peripheral blood of subjects with CD, by means of flow-cytometric techniques. Group A of control was of 20 healthy subjects, aged 5 to 58 years. Study population (Group B) was of twenty-eight patients, all females aged 13 to 70, receiving firstly a CD diagnosis at the SS Annunziata Hospital Digestive Physiopathology Out-standings' by means of clinical, serologic and small intestinal biopsy findings. Peripheral CD34+ HPCs were significantly increased in Group B (median value 0.16) when compared with Group A (median value 0.03) (p 0.0001) but did not correlate either with anti-transglutaminase (tTG) antibody levels (IgA: p 0.226; IgG: p 0.810) or with histological damage severity (p 0.41) that, on the contrary, was significantly related with anti-tTG IgA antibodies (p 0.027). Celiac circulating CD3+CD4+ lymphocytes expressed a chemokine-receptor pattern Th2-skewed in all but three patients investigated. Concluding, the CD34+ HPC highly increased peripheral traffic observed in celiac disease appears more related to a basic and emerging as common defect shared by chronic inflammatory diseases than to the gliadin-specific Th1 local reactions. Data are consistent with a potential NFkappaB deficiency and consequent prevalence of apoptotic versus survival programs leading to excessive cell-death; to replace lost cells a supplementary bone-marrow derived precursors supply, further to that physiologically provided by the gut stem cell "niches" that are cryptopatches, could be required.

Introduction

Immuno-Mediated Inflammatory Diseases (IMID) such as allergic and auto-immune diseases have been interpreted, for more than thirty-five years, as the result of a TH2 cell reaction for allergy and TH1 for auto-immunity respectively, according to a divergent view of TH2/TH1 paradigm, where cell polarization was thought to depend on the nature of antigen/allergen. Doubts concerning such interpretation of the paradigm have thus generated recurrent debate during latest years (1-4).
Later a linear version of the paradigm has been proposed, not only for T-cell lineage but also for NK and dendritic cell (5-11), where H2, H0 and H1 were, simply, the early, intermediate and late stages of an effective cell-lineage differentiation/maturational events (cell-lineage physiological ontogeny). Under this optic the very low amount of TH1 cells characteristic of allergic subjects has been interpreted as the consequence of a disturbed ontogeny due to the difficulty in the progression of differentiation/maturation at the *early/intermediate/late* stages (12-15), sustained by genetic defects impairing the maturational citokine IL12 and IFNs receptors (16-20).
Most recently, T cell-mediated tissue damage has been referred to a novel identified T cell sub-population, termed TH17, generated during an altered T-cell lineage differentiation defective in IL2 and/or IL12 expression (pathway TH17) (21-23) leading to the first major revision of the above mentioned TH2/TH1 hypothesis (24).
TH1 cells, whose role has long been considered paramount in the pathogenesis of autoimmune diseases, are now credited to antagonize Th17 pathway (24).
The large body of evidence regarding monogenic defects underlying a new and wide identified chapter of primary immunodeficiencies, has been systematically reviewed, with special attention to cell and antibody defected maturational processes linked to a disturbed homeostasis of both the innate and adaptive immune systems (20).
Hematopoietic Precursor (HPC) and diverse white-lineages-committed Stem Cell (SC) have been, furthermore, recognised as involved in systemic and local aspects of allergic inflammation (25-41) and their traffic in Peripheral blood (PB) has been shown to be significantly increased in allergic subjects but not during infectious inflammation (42).
The aim of the present study is to investigate the CD34+ HPC and stem cells traffic in PB and the h2/h1 associate pattern of chemokine receptor CXCR4 (H2) and CXCR3 (H1) expression by peripheral T-lymphocytes in subjects suffering from Celiac Disease.

Methods

Subjects

HPC-CD34+ and chemokine-receptor expression have been investigated in PB samples of two groups of subjects, randomly recruited and processed in a blinded manner.
Group A (control) was of twenty healthy subjects (10 males and 10 females aged 5 to 58 years, mean age 24. 5 years) negative for clinical history of any chronic inflammatory diseases.
Group B (pathologic) was of twenty-eight patients (all females aged 13 to 70 years, mean age 32.5 years) receiving the first diagnosis of CD at the *SS Annunziata* Hospital Digestive Pysiopathology Out-standings' by mean of the serological evaluation of anti-gliadin IgG and IgA (AGA-IgG and IgA), anti-trans-glutaminase IgG and IgA (tTG-IgG and IgA) antibodies and of small intestinal biopsy, according with Corazza-Villanacci severity classification (A: increased number of Intra-Epithelial Lymphocytes but no structural modification of the mucosa architecture, B1 = moderate mucosa damage, B2 = severe mucosa damage with not identifiable villous) (43).
Oral informed consent or assent (from under 18 years old) was obtained from the subjects and parents/guardians at the time of recruitment.

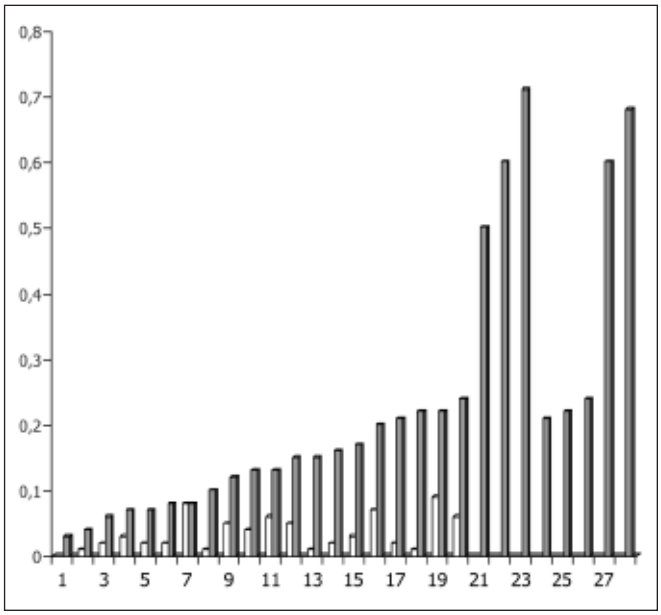
Serological assays

In vitro quantitative measurement of serum IgA and IgG antibodies specific for gliadin has been performed with *ImmunoCap Gliadin IgA/IgG* commercial kit purchased by Phadia AB (Uppsala, Sweden), according to manufacturer recommendations. Results were expressed in mg/l. Data have not been reported since irrelevant.
In vitro quantitative measurement of serum IgA and IgG antibodies specific for human-recombinant tissue transglutaminase (tTG) has been performed with *Celikey* commercial kit purchased by Phadia AB (Uppsala, Sweden), according to manufacturer recommendations. Results were expressed by a quantitative elaboration of examined sample versus a calibration curve. The expression Units/ml were the ratio between $OD_{sample}/OD_{cut\text{-}off}$.
Whit a cut-off value of 6 U/ml clinical sensibility is 96% and clinical specificity is 99%.
Values have been considered as positive from values of 8 U/ml onwards.

Flow cytometry

To investigate the entire pool of immature mononuclear circulating cells, independently from lineage commitment (mono-potent stem cell) or un-commitment (multi-potent hematopoietic precursor cells), a simple method based on a partial modification of the Milan Protocol of Peripheral Blood CD34+ Cell Estimation was followed, as previously described (34, 42).
Peripheral or cord blood venous samples were collected in ethylendiaminetetraacetic acid (EDTA) tubes. Three aliquots of 50 ml of whole blood were incubated, at 4°C for 25 min in the dark, one with 15 μl of phycoerythrin (PE)-conjugated murine monoclonal antibody (MAB) specific to CD34 molecule, one with PE-conjugated MABs specific to unrelated molecules to human leukocyte antigens, and one as unlabeled control. Monoclonal antibodies were from Becton Dickinson (BD), Milan, Italy. PE anti-CD34 MAB was the Anti-HPCA2, Clone 8G12 (BD), suitable for routine use. Then, 2 ml of red blood cell lysis buffer (BD, Italy) were added to each tube and incubate at room temperature for 10 min, in the dark. After lysis cells were washed twice with 2 ml of cold phosphate buffered salt solution (PBS) (BD, Italy) at 1,200 revolution per minute (rpm) (300 g) for 7 min at 4°C and then resuspended in 500 μl of PBS.
Cells were analysed using a FACSCalibur flow cytometer equipped with Cell Quest software. Three data parameters were acquired and stored in list mode files: linear forward scatter (FSC) (vertical axis), linear side-angle scatter (SSC) (horizontal axis) and log PE fluorescence, by gating whole viable cells (Fig.). Since our aim was to enumerate both $CD34^{bright}$ and $CD34^{dim}$ cells, the setting of the fluorescence analysis region 2 (R2) has been fixed with the lower limit at 10^2 avoiding any compensation. For each measurement 10,000 events were acquired. Results have been expressed as percentage of positive cells.
CXCR4 and CXCR3 expression on CD2+CD4+ and CD3+CD4+ cells has been performed using mouse anti-human Monoclonal Antibodies, as follow: CD2 FITC, CD3 PerCP (*Peridinin-Chlorophyll-Protein*), CD4 FITC or PerCP , all purchased by BD.
CD184 PE, clone 12G5, was a mouse IgG2a specific for CXCR4 and CD183 PE, clone 1C6/CXR3, was a mouse IgG1 specific for CXCR3; both monoclonals were supplied by Pharmingen. Cells were processed and analysed by current unmodified three-colour-immuno-fluorescence techniques; results expressed as percentage of cells positive.

Figure 1 - Peripheral blood CD34+ precursor and stem cells are significantly (p 0.0001) increased in celiac disease population (grey bars, 28 subjects, median value 0.16) when compared to control group (white bars, 20 healthy subjects, median values 0.03)



Chemokine receptor (CKR) association with TH-cell polarization

Although none of CKRs can univocally identify TH2 or TH1 polarization, because of the diverse nature of naïve, memory and effector sub-populations, as well as the flexibility of some expression programs, CXCR4/CD184 has been proved to be up-regulated by IL4 (44, 45) and down-regulated by IFNγ (45) and has been described as preferentially associated with TH2 cells (44-46) also in T-cell lymphomas with Th2+ immunophenotype (47).
CXCR3/183 expression, on the contrary, appears to mark the IFN-gamma dependent terminal differentiation processes from naïve to memory cells also in B-lineages (48) and thus has been preferentially associated with a TH1 profile (49, 50), particularly with TH1-activated cells transmigrating into tissue sites of inflammation in autoimmune diseases (51-54).

Statistics

Given the not-normal distribution of continuous variables, data have been processed using non-parametric

tests. Comparison between CD34+ peripheral cells circulating in normal versus pathologic groups has been performed by the *Wilcoxon* signed-rank sum test for independent samples. Relationships potential between peripheral CD34+ cell increased traffic and serum auto-antibodies specific to Gliadin, Transglutaminase have been explored using *Spearman*'s rank correlation and with histological damage of the intestinal mucosa using χ^2– test. A p value < 0.05 was considered statistically significant.

Results

CD34+ cell values in PB of healthy subjects (Group A) ranged from 0.01% to 0.09% with a median of 0.03. CD34+ cell values in PB of subjects with Celiac Disease (Group B) ranged from 0.03% to 0.70% with a median of 0.16 and thus resulted significantly increased ($p<0.0001$) when compared with (Group A) (Fig. 1).
Comparisons between Group B CD34+ cells and serum anti-tTG IgA and IgG values were not significant since rank-correlation resulted 0.233 ($p = 0.226$) and 0.051 ($p = 0.810$), respectively.
Equally, χ^2 – test between CD34% categorised with two values major of the median (16) and minor versus histological damage severity [A vs (B1 and B2)] resulted not significant with a $p = 0.41$.
Spearman's rank correlation between serum IgG-anti-tTG and tissue damage (histology severity score) was negative ($p = 0.534$) while IgA-anti-tTG correlated significantly ($p = 0.027$) with grade B of mucosal damage.

The expression of peripheral pattern of chemokine receptors, among CD4+ lymphoid populations (T-cell + a subset of Natural Killer) marked by CD2, was always skewed to h2 (CXCR4) with the exception of one subject . Among CD3+CD4+ T-cell population only three subjects showed a percentage of cells expressing the h1/associated receptor CXCR3 major than the h2/associated (CXCR4) (Fig. 2).

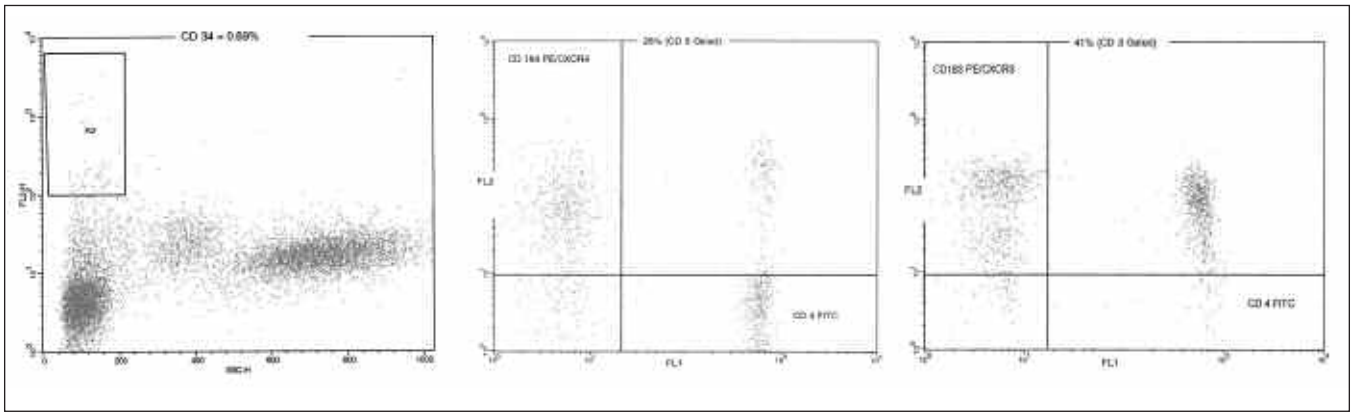
Discussion

Results from the present work have to be discussed in a complicate and fast moving background involving physio-anatomical, immunological and epithelial homeostatic mechanisms.

Stem cell niches in mammals

Accumulating evidence indicates that almost every adult tissue contains stem cells clarifying how cells and microenvironment operate to maintain homeostasis in self-renewal tissues.
Bone-marrow, once regarded as the major if the unique source of HPC and stem cell in the body, has been recently proposed to be a reservoirs of stem cell populations beside the local stem cell niches (55-58).
Gastrointestinal stem cell niches provide, under normal condition, to the physiological replacement of epithelial cell lineages undergoing to a high-rated turnover. Stem cells reside, mainly, in crypts that represent the proliferat-

Figure 2 - Even if celiac patients had a peripheral lymphocyte CD3+CD4+ pattern of KCRs expression prevalently Th2-associated, CD34+HPCs moved significantly also when peripheral T-cells were Th1-polarised, as in the case showed. From left: % of PB CD34+ mononuclear cells, CD3+CD4+ CD184+ (CXCR4 H2-associated), CD3+CD4+ 183+ (CXCR3 H1-associated)



ing compartment when villous the differentiation one, receiving cells from crypts (59, 60). A bone marrow contribution to regeneration of intestinal myofibroblasts has been described during epithelial reparative processes (60-62).

The gut as extra-thymic site of lymphocyte differentiation

After the first report of T-cell maturation in the human gut by Marie Louise Hammarstrom et al. in 1995 (63) and the consequent initial controversies, a large and compelling body of evidence has been cumulated to regard gut as the Thymus substitute-complementary organ for T-lymphocyte precursors development and selection (64-69). In absence of thymopoiesis, in fact, T-cells are nevertheless present principally in gut. The elegant model of athymic/euthymic mice furthermore indicates that in euthymic animals the alternative lymphopoietic pathways are repressed except in conditions of severe lymphocyte depletion (69, 70).
The question about the origin of the lymphoid precursors subsequently arose. In human gastrointestinal tract, hemopoietic stem cells (HSC) CD34+ CD45+ were detected both in the lamina propria and in the epithelium, localized in crypts and in villous.
Most of the HPC were CD34+, CD45+ but c-Kit negative, indicating ongoing differentiation and expressed the CD7 marker of lymphoid lineages. Almost 50% HSC were positive for CD56 in the epithelial layer when only few in lamina propria, suggesting that gut epithelium could be a preferential site of lymphoid Natural Killer (NK) differentiation (71).
Recently the issue has been extensively reviewed (72, 73), with a special attention to the subset of IntraEpithelial Lymphocytes (IELs) that constitute a unique population expressing the T-cell receptor (TcR) alpha beta or gamma delta but CD8 alpha alpha homodimers. Such a T-cell subset is believed to be regulatory, to appear downstream the transitional double positive population CD4+CD8+ (DP) and that could originate by immature but lymphocyte-lineage committed thymic emigrant cells in euthymic mice or by HSC harboured in cryptopatches in athymic animal (74).
Gut criptopatches thus appear to constitute a major component of a physiological system capable of sustaining the entire ontogeny of T-cell lineages phylogenetically less evolved with respect to lineages educated in the thymus.
Thymus-derived cells may have selected to process foreign antigens bearing a wide diversity of TcR diversity and are particularly active in youth when extrathymic T-cell lineages appear to recognize self-reactive abnormal cells and act mainly as immunoregulatory populations in site exposed to antigenic overload. Extrathymic T-cells are few in youth but increase with aging in parallel with thymic involution. Under condition of lymphocytes deficiency as stress and pregnancy this compartment appears expanded even in youth. Taken together mentioned features suggest that T-cells associated with the small intestine epithelium could represent the ultimate evolutionary step of innate immunity bordering and supporting adaptive immunity (75, 76).

Cell-mediated immunological tolerance at glance

The identification of different subsets of regulatory lymphocyte as well as of different cellular signalling molecules and related pathways could be considered one of the most important progress in a field crowded of actors.
Establishment of central tolerance critically depends on AIRE (autoimmune regulator gene) transcriptional activity that allows the thymic medullary epithelial cells (TEC) expression of tissue-restricted self-antigens and the consequent negative selection of the T-repertoire thorough clonal deletions of auto-reactive T-cells, before thymic egression. The developmental expression of AIRE final products on mature medullary TEC appears linked to the cell effective differentiation/maturation dependents on non-classic members of the IkappaB inhibitors family that contrast apoptotic signals at the intermediate stage of the development. Perturbing such a signals pathway has been reported to be associated with a breakdown of central immune tolerance (77-80).
Peripheral tolerance is more complex because of an excessive suppressor activity leads to immunodeficiency or cancer whilst a loss of the function generate autoimmunity; maintaining homeostasis thus requires a perfect balance between action and resting.
Several types of T-regulatory cells (Treg) have been described in last decades generating difficulties in the comprehension of their activity. Among the many, the best characterized are those residing in CD4+ subset that are CD4+CD25+ Treg and type 1 regulatory (Tr1) cells.
Tregs are natural occurring cells generated mainly in the thymus, displaying a highly diverse TcR repertoire (81) and acting in a singular fashion because their activation is antigen-specific but inhibition of effector T-cells is antigen-not-specific (bystander) and cytokine-independent but mediated thorough cell-cell contact (81-84) .
Tregs have been also studied in cord blood (85, 86) rising

the question about a major potential role of such cells in the tolerance induced by CD34+ stem cells (87, 88).
The major feature in CD4+CD25+ Treg subset is the expression of the forkhead transcription factor Foxp3 (Forkhead box P3); interestingly such box has been identified in adenocarcinoma cells as capable of inducing anergy of co-culture T-cell as possible mechanism of cancer immune evasion (89).
Tregs suppress a variety of response including CD4 and CD8 cell proliferation, IFNgamma production, CTL activity (83-85) and appear to regulate NK activity, hampering the generation of mature forms that together with immature dendritic cells guarantee at the periphery a quiescent status of the immune cells (90).
When receptors belonging to the Toll Like family (TLRs) are engaged by pathogens, Tregs are down-modulated allowing an effective immune reaction (91, 92).
Type 1 regulatory cells (Tr1). Opposite to natural occurring Tregs up-regulated by H2 cytokines IL4 and IL13 (82) or by TGF-beta + IL2 (83) and operating suppression in a bystander mode, Tr1 appears a more differentiated cellular subset induced by antigen stimulation and IL10 secreted by immature dendritic cells (DC2); Tr1 regulatory activity is Ag-specific and mediated by IL10 production and thus such cells have been defined *adaptive* to render the concept of specific-suppression even if T-cell Receptor, once expressed, does not undergo further modification as B-cell-receptors do (93, 94). Tr1 population thus appears to be the major candidate as peripherally induced antigen/allergen specific suppressor cells generated, in a tolerogenic environment, with effective low-dose regimens of specific immunotherapy.
A third regulatory population, apparently distinct, but closely related with Tregs and Tr1, is the Th3 subset. Th3 cells are characterized by the production of TGF-beta, and exert their suppressor activity via TGF-beta secretion as well as Tr1 via IL10 secretion (95-97).
Whether Tregs, Tr1 and Th3 cells are three distinct T-subsets with distinct genetic programs or, on the other hand, they represent downstream products of a plastic T-cell lineage proceeding into sequential developmental stages allowed by apoptosis repression induced by different extrinsic co-stimulators (local microenvironment cytokines), it is matter of future evidence when more data from human will be available.
Singularly, human CD25+ regulatory cells express alpha4beta-integrin as homing receptors (98) as well as cord blood CD34+ HPCs (99) and short developmental program cell lineage such as mast cells (100).

Natural Killer T subsets and their regulatory activity: NK-regs

The positive answering to the recently posed question: do "NK-reg cells" exist ?" (100) is from evidence cumulated during the last years.
In 1996, Researchers from The Jefferson M.C. of Philadelphia, described a functionally immature human NK subset, not cytotoxic, expressing only the NKR-P1A receptor but negative for all the other differentiation antigens of mature cells (NKR-P1A+ CD56- CD16-); this subset developed the effector functions when stimulated with IL12, concomitantly with the expression of CD56 (102). Exactly ten years later data have been confirmed by Stanford Researchers (103).
A more accurate knowledge of NK cells immunobiology has recently reappointed the attention over a paramount potential of such a Lymphoid subsets in maintaining immune homeostasis.
Ontogenetic studies suggest that NK progeny rising from CD34+/Lin- precursors, in absence of cytokine/cell-contact specific stimuli, expresses Killer Immunoglobulinlike Receptors (KIR) and is negative for the lectin-receptor CD94 (104), evidencing a non-random sequence of receptor acquisition. IL12 stimulation (105), in fact, induces the expression of the CD94/NKG2-A that is an inhibitory receptor with respect to the NKG2-D, among the products of several gene-subfamilies of lectin-like receptors associated with NK gene complex located on chromosome 12.
The CD94/NKG2 family of receptors is composed of members with activating (NKG2-D) or inhibitory (NKG2-A) capabilities and initial information from clinical condition of excess of tolerance or stimulation is growing in literature (106). NK cells from chronic HCV subjects have been reported to be skewed toward a CD94/NKG2A expression with respect to normal controls and incapable of inducing differentiating changes on immature dendritic cells (iDC) necessary to sustain an effective HCV-clearance (107).
On the other hand, while a direct role for NKG2D-activating receptor has been proposed for CD villous atropy (108, 109), a deficiency of the gut NK population bearing the inhibitory receptor has been associated with experimental autoimmune diabetes (110).
Of major interest is, furthermore, the highly significant ($p < 0.001$) deficiency of a particular *invariant NKT-subsets*, recently reported in Crohn's disease and ulcerative colitis (111), and in celiac disease (112); whilst the role potential of iNKT cells has been matter of controversies with

respect to asthma and allergy (113), evidence suggests a constant reduction in bowel inflammatory disease.
Invariant NK T-cells have been reported, paired to the alpha 14/24 chain, to use the Vbeta 8.2 chain (114); singularly, the Vbeta 8.2 + subset resulted the unique significantly reduced among the Vbeta-repertoire of T circulating cells of a symptomatic mite-allergic population (115), raising the question as to whether such a defect may represent a common feature of immune-mediated inflammatory condition.
Since it is known that germline transcription of the unrearranged Vbeta 8.2 gene is an early event of lymphocyte development, at the stage of precursors, long before Vbeta families rearrangement (116), it has been recently proposed that early lymphoid cells could use a pre-TcR structure based on Vbeta 8.2 gene espression (117).
The ontogeny and function of iNKT are strictly dependent on NF-KappaB signalling (118). Maturation of iNKT precursors depends on the NF-kappa B induced activation of survival program (e.i. up-regulation of Bcl-2) and repression of apoptotic program (e. i. down-regulation of Fas) (119, 120).

Toll-Like Receptors and NF-kappaB/IkappaB regulatory system

NF-kappaB controls a network of genes including those for immune response, cell adhesion, differentiation, proliferation, apoptosis and angiogenesis.
Phylogenetically ancestral, NF-kappaB has been perfectly conserved through evolution as an evidence for a master role in the regulation of the many biological activities above mentioned.
Resting in normal condition because constantly controlled by its inhibitor I-Kappa-B, an activation of the transcription factor NF-kappaB is allowed by the ubiquitin-dependent degradation of the inhibitor I-kappa-B, induced by stimulus-dependent phosphorylation (121, 122), on the analogy with other biological enzymatic cascade pathways like, e. i. the *Complement* that is upstream controlled by the C1q-inhibitor (C1qINH).
A disruption of the mechanisms regulating the NFkappaB and IkappaB balance has been proposed as related to the development of many immune mediate inflammatory diseases (123).
The Toll-like receptors (TLRs) belong to a type I integral membrane receptors recognizing pathogen associated molecular patterns (PAMPs) that are conserved microbe derived molecules.
Diverse TLRs can recognize diverse molecules from microbial cell-wall or can recognize viral RNA and bacterial DNA and thus, despite invariant, constitute a restricted repertoire of diversity recognition.
If mainly investigated for their paramount role in the activation of innate immunity and, as emerging, as critical controller of the acquired immunity where different TLRs can have different effects, TLRs are broadly distributed on a variety of tissue. Particular interest is emerging in studies about epithelial TLRs distribution and function that together with NF-kappaB defect could be of some importance in the discussion of our data in the contest of celiac disease.
Among the many functions, NF-KappaB exerts an active control on apoptotic balance; very recently it has been reported that NF-kB deficiency leads to apoptosis of colonic epithelial cells and to the development of a chronic inflammatory response, involving at the beginning cells of the innate immunity and later T-cells, similar to the histopathology of inflammatory bowel diseases (124).
Another emerging mechanism involved into colonic homeostasis appears linked to the anatomical location of Toll-like receptors with a special role for TLR9.
Among the several TLRs expressed on the cell surfaces of intestinal epithelium, TLR9 appears to deliver inhibitory signal on NF-kappaB when located at the cell apex whereas activating signal if basolaterally located (125-127).

Data discussion

Hematopoietic Precursor and stem cell CD34+ circulating in the peripheral blood of patients suffering from celiac disease resulted highly and significantly ($p< 0.0001$) increased with respect to healthy population.
Such a feature has been well investigated in allergic diseases (25-42), particularly in asthma, also as a consequence of allergen challenge and production of stromal-cell derived factor 1 alpha (SDF-1 alpha) that is capable of inducing a bone marrow (BM) CD34+ mobilization (39) or to investigate mechanisms underlying bronchial remodelling (128) or other conditions, such as heart stroke in which CD34+ HPC are involved in post-lesion reparative fibrosis (129) or during gut epithelial reparative processes (61, 62). Nevertheless, as myofibroblasts move just at the beginning of asthma inflammation, at the mild intermittent step, and thus appear to induce fibrosis without anatomical injuries, also in CD patients the increased

traffic of CD34+ HPC resulted not significantly related ($p = 0.41$) with intestinal epithelial damage as assessed by biopsy, underscoring an interpretation as a possible ongoing reparative mechanism.

A discussion related to the emerging function of the gut as a primary immune organ, as suggested by the compelling evidence briefly reviewed, is intriguing not only for CD pathogenetic implications but also for the aspect potential regarding the peripheral tolerance failure.

In fact, if central tolerance is a matter for the Thymus, peripheral immune homeostasis, particularly on adulthood after thymus involution, appears to involve gut associated lymphoid tissue more and more.

Furthermore, a better knowledge of the molecular signalling pathways that regulate the development of different cell subpopulations with different activities on the delicate equilibrium between anergy-tolerance and action sustaining the immune homeostasis may allow a view more consistent of some clinical observations previously considered paradoxical.

From the above mentioned evidence two fundamental elements rise: the first is in that *tolerance/hyporesponsiveness* appears a function proper of the *immature* cells belonging to a committed lineage when the acquisition of *effector* functions is a consequence of the transcription of the differentiation program along the lineage.

The second, that could explain the why of the reported increased traffic of CD34+HPC, is the mechanism that maintains anergy or allow the progression towards the effector stages that is based on cell death.

Apoptotic balance is under the active control of NfkappaB, as reported (119-124), that activated on demand, down-regulates cellular death allowing survival programs required for the differentiation.

Such a model suggests that on resting condition hyporesponsiveness/anergy is maintained by a controlled-cell death of the immature compartment (H2) that thus requires a constant incoming of immature precursor cells necessary to replace the programmed loss.

Under physiological conditions the HPC supply may be guaranteed by the local reservoirs that are niches and crypto-patches without any bone-marrow contribution (CD34+ HPC peripheral circulation in normal subjects is very low if absent); a defect afflicting NFkappaB/IkappaB activity, as reported (123, 124), may results in an excessive high-rate of apoptosis that induces an HPC extra-demand that requires bone-marrow mobilization and the observed increased peripheral traffic of CD34+ HPC (Fig. 3).

A quite constant pattern skewed to TH2 conditions, observed at the circulating cell compartment, also in TH1 locally dominated diseases, could be consistent with the emerging scenario.

Celiac disease could represent a perfect model where an expansion of the immature-cell compartment sustains a final TH1/TH17, antigen-specific (gliadin), autoimmune reaction.

Our data, in fact, show an overwhelming H2-oriented lymphoid pattern in the peripheral blood and, even in the three cases where H1 pattern prevailed, the CD34+ values were very high.

A coexistence between TH2 and TH1 behaviours (130-132) could be thus explained on the model that physiological response is TH2 and becomes TH1 when survival programs switched on by extrinsic stimuli allow the differentiation progress; under not-physiological conditions an expanded H2 compartment may receive persistent survival signals by extrinsic antigens such as gliadin. Thus, anatomically, we could identify H2 bearing markers cells at the crypto-patches level where differentiating processes involve CD34+ precursors and stem cells and H1 cells at the villous level, with intermediate patterns of cytokine secretion (IL10, TGF-beta) detectable along the differentiation/maturation pathway.

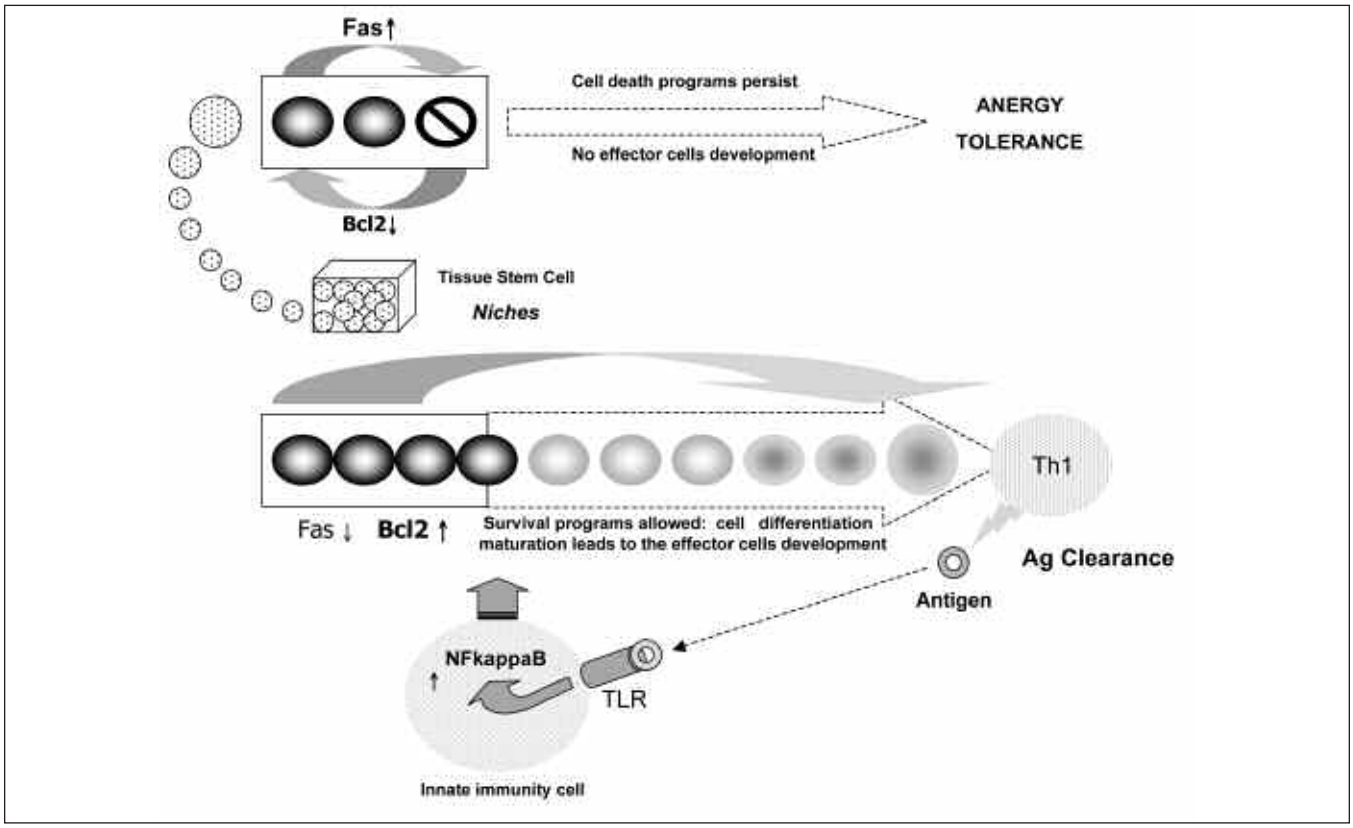
Among the regulatory cell populations if poor evidence exists for a Tregs defect in CD (133), a large body of experimental data suggests a disturbed homeostasis of the lymphoid NK subsets to play a central role not only in celiac disease but also in the determinism potential of many of the other chronic inflammatory frequently associated diseases.

The acquisition that different suppressor/stimulating properties depend on the progression of the cell differentiation/maturation pathway has been well defined for NK-lineages as well as defects of the regulatory systems are going to be clarified.

Such studies evidenced as developmental stages close to lineage-precursors (immature cells) display suppressor/anergic function when modulating properties are acquired at the intermediate stages to become effector cells at the ultimate steps of the maturational process; the H2 – H0 – H1 linear version, originally developed for educational purposes, results useful to describe the pattern of cytokine and chemokine associated with the different developmental/functional status.

Two elements emerged as critically controllers of the differentiation progress from lineage precursors onwards: IL12 and NFkappaB (101-120).

Figure 3 - Emerging potential model of lymphoid lineages homeostasis, as suggested by recent evidence. In absence of antigenic stimuli, immature cells can not differentiate because subjected to apoptotic programs that guarantee hypo-responsiveness/anergy. Under physiological conditions, lost cells are replaced by precursors locally harboured in the tissue *niches* (upper section) and thus CD34+ traffic in peripheral blood is very low if absent. When an antigen has to be removed, it is firstly engaged by toll-receptors of cells belonging to innate immunity with a consequent activation of NFkappaB. Apoptosis is then down-regulated and survival programs allow the proceeding of the maturational events leading to the differentiation of effector Ag-specific Th1 cells. NFkappaB deficiency thus, could reduce the effector Th1 cell development resulting in a immunodeficiency. Permanent up-regulation of apoptotic programs, furthermore, may induce an excessive cell death that can not be replaced by the exhausted local *niches* requiring an extra-supply of bone-marrow derived circulating precursor and stem cells



Since it is recognized that IL12 gene is under NFkappaB control, a defect of the latter appears capable of disregulating the entire lineage ontogeny from the beginning.

A deficiency of NFkappaB activity in fact, is necessary not only for the repression of apoptosis and the allowing of survival programs but also for the transcription of IL12, essential for the expression of the receptor regulatory family CD94/NKG2 A-G.

We have previously stressed the attention on the reported deficiency in inflammatory bowel diseases of a particular invariant NKT cell population (iNKT) (111, 112).

The main features of such iNKT subset are suppressive action, an early developmental status close to the precursors (116) and the expression of a truncated-like receptor (pre-TcR) using the Vbeta 8.2 chain not rearranged but germline encoded (116, 117).

The reported deficiency of a lymphoid subset Vbeta 8.2+ also in active allergic respiratory disease (115) could suggest that such deficiency may represent a defect shared by respiratory and digestive immune-mediated inflammatory diseases. Since the Vbeta 8.2+ deficiency was absent in an allergic population asymptomatic after an effective regimen of low-doses allergen-specific sublingual immunotherapy, a low-dose gliadin SLIT expanding gliadin-specific Tr1 IL10-producing-cells may represent the most convenient approach to restore gliadin tolerance (134) with respect to other experimental approach of oral tolerance induction based on the ad-

ministration of adjuvant-coupled antigen, expanding FoxP3+ Tregs (135).

A NFkappaB deficiency, furthermore, not only induces an exaggerated apoptosis rate of the lymphoid immature cell compartment but, equally, of the intestinal epithelial cells (124) and it is conceivable that the anatomo-physiological sources of precursors necessary to the normal-rated cellular turnover, that are crypto-patches, are incapable of supporting the excessive-rated cellular death leading to the epithelial progressive destruction.

Auto-antibodies of A class to transglutaminase, that are the only parameter significantly correlated (p= 0.02) with histological damage, could furthermore worsen NFkappaB deficiency. Tranglutaminase, in fact, has been reported to enhance NFkappaB activity by the polymerisation of the inhibitor IkappaB (136).

Celiac disease, thus, represents an experiment of nature from which many lessons could be learned.

It has a component depending on Th1-antigen specific reaction that characterise the inflammation at the villous and that is well controlled by a strict gluten-free diet; furthermore NFkappaB deficiency may induce an abnormal death rate of cell belonging to the immature compartment where evidence suggests to reside regulatory subsets.

Such a regulatory populations deficiency could affect not only the local gliadin-specific reaction but also peripheral tolerance, mainly in the adulthood when thymus regulatory activity declines.

A reduction of suppressor cells systemic activity, particularly of the NKT-lineage, whose physiological ontogeny appears linked to the GALT function as primary lymphoid tissue, could thus underlie the frequent association of CD with allergic and autoimmune diseases.

Under this optic, the highly significant (p< 0.0001) increased peripheral traffic of CD34+ precursor and stem cells appears more related to a compensatory mechanism consequent to the excessive cell-death rate due to the NFkappaB malfunction rather than to the gluten-specific Th1 reaction itself.

In other words, an increased input of CD34+ HPC in a gut where the anatomical segregation of the immature cells into crypto-patches is disrupted because of the compromised epithelial barrier, may result in an activation of such immature cells by the polyclonal activators largely represented in common foods, raising the question as to whether in Refractory Celiac Disease (137) a diet free only of gluten could be still regarded as adequate.

Concluding, meanwhile protocols devoted to expand gluten-specific regulatory-cells will be developed as well as synthetic agonists of the diverse Toll-like receptors will be better investigated (138, 139), an attempt to limit NFkappaB inefficiency by means of the stimulation of different TLRs using probiotical formula containing more than one bacterial strain may be considered.

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2009 WAO Congress

The World Allergy Organization (WAO) holds its World Allergy Congress (WAC) every two years.
Each Congress attracts over 4.000 experts and scientists working and interested in the fields of allergy, immunology and other related fields.

2009 WAO Congress
Buenos Aires, Argentina
Dates: 6-10 December 2009
Website: www.worldallergy2009.com

STALLERGENES
Allergen vaccines worldwide

Desensitisation:
treating allergy at its origin

Every day we make advances in treating respiratory allergic diseases, improving the quality of life of patients

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Press release: Sublingual desensitisation tablets Oralair® Grasses approved in Germany

Antony, France; June 24th, 2008 – Today, Stallergenes has been granted with the marketing authorisation for its sublingual desensitisation tablet, Oralair® Grasses in adults, by PEI (Paul Ehrlich Institute), the biological branch of the German health agency.
Based on the positive results of the paediatric pivotal study (VO52), Stallergenes will immediately apply for a paediatric extension of the product's indications.
Stallergenes will launch Oralair® Grasses in the adult indication on the German market in the coming weeks. The company expects to extend this launch to the paediatric population this year so as to capture the forthcoming prescription period for grass pollen desensitisation in both indications.
Subsequent market authorization in Europe will be applied afterwards through a Mutual Recognition Procedure (MRP) for both indications, Germany being the reference member state.
"The registration of Oralair® Grasses in Germany is a significant breakthrough that gives desensitisation treatments the same level of recognition as mainstream pharmaceuticals and creates a new therapeutic class: "the allergens". I am particularly proud of the fact that Stallergenes has succeeded in turning itself into a genuine biopharmaceutical company, capable of developing and registering pharmaceuticals that meet the highest regulatory standards. We expect the Oralair programme to bring in a steady flow of product licenses in the next few years," says Albert Saporta, Chairman and CEO of Stallergenes.

About the Oralair development programme

According to World Health Organisation (WHO) estimates, 20 to 25% of the world's population suffer from respiratory allergic symptoms, rhinitis and/or asthma. By 2020, 50% of the world's population will be concerned by allergy according to ISAAC study. According to WHO, desensitisation is the only treatment that addresses the immunological cause of allergy and modifies the natural course of the disease. Nearly 15 to 20% of these patients suffer from moderate to severe allergic rhinitis and rhino-conjunctivitis, not controlled by their usual medical treatment.
Since 2003, Stallergenes has been carrying out the Oralair programme which addresses these unmet medical needs with EBM-documented, registered allergen tablets that are safe and easyto- use.
This programme consists in the development of the four main allergens accounting for more than 80% of the epidemiology of these allergies: grass pollens, house dust mites, birch pollen and ragweed pollen.
The entire programme is in the clinical development stage and is proceeding according to schedule.

About Oralair® Grasses

With a safe and easy-to-use daily dose of Oralair® Grasses, patients enjoy a very significant alleviation of all their rhino-conjunctivitis symptoms, a marked reduction in their symptomatic medication use and a noticeable improvement in their quality of life.
Oralair® Grasses is a fast-dissolving tablet that has demonstrated high efficacy in allergic rhinoconjunctivitis to grass pollen in the first season, throughout the pollen season and at the pollen peak on:
- poly- and mono-sensitised patients, as well as asthmatic patients,
- every individual symptom, and in peculiar in nasal congestion and watery eyes

Oralair® Grasses is a pre-coseasonal treatment: it has to be started four months before the pollen season, maintained throughout the season, then stopped and restarted the following season.
Oralair® Grasses contains a mix of 5 standardised grass allergens: perennial rye grass (*Lolium perenne*), meadow grass (*Poa pratensis*), timothy grass (*Phleum pratense*), cocksfoot (*Dactylis glomerata*) and sweet vernal grass (*Anthoxanthum odoratum*), as a daily dose of 300 IR, so as to mimic patients' natural exposure.
The clinical development programme has already enrolled around 1600 patients to date. A long term pivotal study is proceeding according to schedule, and is currently in its second year. The company plans to file two INDs for adult and paediatric trials with the FDA this year.

About Stallergenes

Stallergenes is a European biopharmaceutical company dedicated to desensitisation therapies for the prevention and treatment of allergy-related respiratory diseases, e.g. rhino-conjunctivitis and allergic asthma. A pioneer and leader in sublingual desensitisation treatments, Stallergenes devotes 18% of its turnover to Research and Development and is actively involved in the development of a new therapeutic class: sublingual desensitisation tablets.
In 2007, Stallergenes had a turnover of 147 million euros and provided desensitisation treatments to more than 500,000 patients.
Stallergenes is listed in Euronext Paris (Compartment B) and is part of the sample composing the SBF 120 index.

ISIN Code: FR0000065674
Reuters Code: GEN.PA
Bloomberg Code: GEN.FP

Additional information is available on http://www.stallergenes.com

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ImmunoCAP ISAC, an innovative tool in allergy diagnosis

Promotional article sponsored by Phadia SAS

Allergy *in vitro* diagnostic tools

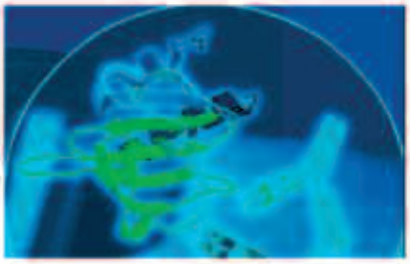
Diagnosis of type I allergy is based on anamnesis, provocation testing, and serological determination of total and specific IgE. Historically available standard products for *in vitro* or *in vivo* allergy testing have consisted in allergen extracts prepared from biological raw materials (e.g., pollen, mites, animal dander, moulds, foods, venoms, etc.). They consist of natural mixtures of allergenic and non-allergenic molecules which respective representativeness in the final product has ever been a challenge successfully took up by Phadia. However, the need for increased specificity resulting from a better knowledge of the composition of allergenic sources has been satisfied by the recent development of tests specific for allergen components obtained thanks to genetic engineering or extensive purification.

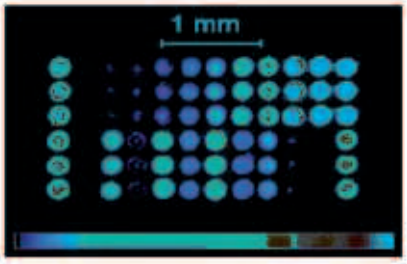
Component resolved Diagnosis

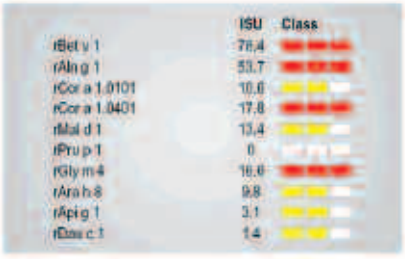
Highly cross-reactive allergen components are present in many biological sources, as an example profilin is found in a broad variety of plant pollens and plant derived foods. A sensitisation towards such a panallergen creates positive test results against numerous allergen extracts. Consequently, when using extract based specific IgE testing it may be difficult to identify the correct allergen source, more especially when cross-reactive allergen components are involved.
The application of recombinant DNA technology to the field of allergen characterization has allowed revealing the molecular nature of the most common allergens. The use of allergen components instead of crude, natural extracts for allergy diagnosis provides each patient with its individual IgE reactivity profile. The knowledge of which components are involved – specific marker or indicating crossreactivity – is the starting point for more informative and powerful diagnostics. Today, the increasing availability of allergen components, either recombinant or purified natural, leads to the way for a new era in allergy diagnosis and gradual transition towards Component Resolved Diagnostic (CRD).
Phadia already offers more than 60 individual allergen components for *in vitro* allergy testing with the ImmunoCAP technology. However, as some patients develop very complex allergen specific IgE sensitization patterns, a new type of serological test exploring a larger number of individual allergens is required for a comprehensive analysis of their IgE binding pattern.

ImmunoCAP® ISAC

In the recent years, the originally DNA focused biochip technology has been adapted for the monitoring of multiple binding events on a protein level. The benefits of this innovative approach are the minute sample volumes, the high sensitivity and the enormous number of measurements conceivable in a single drop of sample liquid, e.g. human blood.
VBC Genomics and Phadia have combined this innovative biochip technology with cutting-edge research in molecular allergology to develop a novel diagnostic test, ImmunoCAP® ISAC (Immuno Solid-phase Allergen Chip). This miniaturized immunoassay allows for multiplex measurement of specific IgE antibodies to many purified natural or recombinant allergen components using only 20 µl of serum or plasma.
ImmunoCAP® ISAC is the first multiplex *in vitro* diagnostic tool for the allergy specialist that is based exclu-



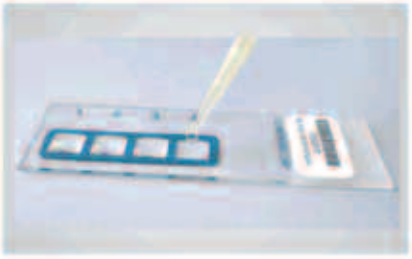


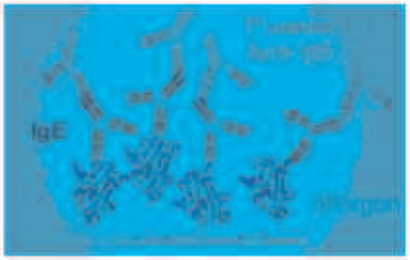


sively on allergen components. The test detects IgE antibodies related to grass- or tree-pollen, animal dander, food, insect venom and mould allergens, amongst others. To date, 103 allergens are included in ImmunoCAP® ISAC, covering a broad spectrum of different allergies. ImmunoCAP® ISAC allows a better understanding of the IgE-reactivity profile and leads to the improvement of the diagnosis and the treatment of patients with complex patterns of allergen specific IgE sensitizations.

Advantages of ImmunoCAP® ISAC technology

- Multiplex specific IgE measurement to allergen components from over 40 common allergen sources in a single test.
- Component resolved diagnosis (CRD) using only purified natural or recombinant allergen components.
- Marker allergen components – specific and indicating cross-reactivity.
- Semi-quantitative results based on fluorescence measurements.
- High reliability by intrinsic replicate testing and quality controls.
- 20 µl of patient serum/plasma give complete results.
- Capillary blood sampling enables less invasive procedure for testing young children.





News

American Academy of Allergy Asthma & Immunology (AAAAI) - For immediate release, February 5, 2008

Spring cleaning: It´s time to clean up indoor allergens
MILWAUKEE-For the more than 40 million people throughout the country who suffer from indoor allergies, spring cleaning can be an important step to take in order to reduce allergy symptoms, according to the American Academy of Allergy, Asthma & Immunology (AAAAI). Spring cleaning takes some time and effort, but it will produce an indoor environment that is less allergenic, easier to clean and healthier for the whole family.

Symptoms of indoor allergies
Indoor allergy sufferers will often wheeze, sneeze, cough and hack their way through the winter months, thinking they have a chronic cold. In actuality, they are probably reacting to indoor allergens. Some symptoms between a cold and allergies are similar, such as sneezing and a stuffy or runny nose. But, if your symptoms are also accompanied with a fever, sore throat, colored nasal discharge, and aches and pains, then you probably have a cold. With allergies, there is never a fever, the nasal discharge is clear, and eyes may become red and itchy. Furthermore, while a cold usually lasts about a week, allergies can last all year.

Prevention of common indoor allergens
The key is to focus on sites where allergens accumulate. The term "allergen" refers to any substance that can trigger an allergic response. First, you must know which allergens or irritants in your home provoke your symptoms. Common allergens and some ways to prevent them include:

Dust mites: These thrive in house dust, which is composed of plant and animal material. Their droppings are the most common trigger of perennial allergy and asthma symptoms. Change and clean cooling and heating system filters once a month.
- Have your home, car and office vacuumed and dusted frequently.
- Wash blankets and bedspreads weekly and sheets and pillowcases more often. Be sure that the water is above 130 degrees.
- Try to regularly wash your curtains and throw rugs.

Molds: These are microscopic fungi. Their spores float in the air like pollen and are present throughout the year in many states. Molds can be found indoors in attics, basements, bathrooms, refrigerators and other food storage areas, garbage containers, carpets and upholstery.
- Keep bathroom and kitchen surfaces dry, fix leaky plumbing and seal cracks where water can seep in to avoid mold buildup.
- Never put carpeting on concrete or damp floors, and avoid storing clothes, papers or other items in damp areas.
- Reduce humidity in damp areas by using a dehumidifier. Clean dehumidifiers once a week.
- All rooms, especially basements, bathrooms and kitchens, require ventilation and consistent cleaning to deter mold and mildew growth. Use a cleaning solution containing 5% bleach and a small amount of detergent.

Pets: People are not allergic to their pets´ hair, but to a protein found in the saliva, dander (dead skin flakes) or urine of an animal with fur. These proteins are carried in the air on small, invisible particles and can trigger allergy symptoms.
- If you have a cat or dog, it might help reduce household allergens by washing your pet once a week.
- Do not sleep with your pet. Sleeping with your pet, long or short-haired, greatly increases the amount of contact with unwanted allergens.
- Vacuum and mop your floors regularly to remove excess animal dander.

Cockroaches: These live in warm, tropical climates, but various species dwell in the offices and homes of humans living in various climates. A protein found in their droppings can trigger allergy and asthma symptoms.
- Frequently remove all household food wastes, including

garbage and recyclables. Food should be stored in sealed containers.
- Wash dishes immediately after use in hot, soapy water, and clean under stoves, refrigerators or toasters where loose crumbs can accumulate. Wipe off the stove top and clean other kitchen surfaces and cupboards regularly.
- Consider a professional exterminator to eliminate cockroaches.
- Thoroughly and frequently clean to remove dust and cockroach byproducts.

When should you see an allergist/immunologist?
By conducting a thorough history of your health and performing allergy tests, if needed, an allergist/immunologist can help you determine which indoor allergens provoke your symptoms. Environmental control measures differ for dust mites, animal allergens, cockroaches and molds, but your allergist/immunologist can help you determine ways to reduce your exposure to these allergens. To relieve your symptoms, your allergist/immunologist may also prescribe appropriate medications, such as antihistamines, decongestants or asthma medications and allergy vaccine therapy (immunotherapy). Visit www.aaaai.org for more information on indoor allergens.
The AAAAI represents allergists, asthma specialists, clinical immunologists, allied health professionals and others with a special interest in the research and treatment of allergic disease. Allergy/immunology specialists are pediatric or internal medicine physicians who have elected an additional two years of training to become specialized in the treatment of asthma, allergy and immunologic disease. Established in 1943, the AAAAI has more than 6,500 members in the United States, Canada and 60 other countries. The AAAAI serves as an advocate to the public by providing educational information through its Web site at www.aaaai.org.

American Academy of Allergy Asthma & Immunology (AAAAI) -May 5, 2008

Asthma attacks early in pregnancy put baby at greater risk of birth defects, new research suggests
MILWAUKEE -Uncontrolled asthma during the first trimester of pregnancy greatly increases the risk of birth defects in babies, according to new research to be published in the June issue of the Journal of Allergy and Clinical Immunology.
Canadian researchers Lucie Blais, PhD, and Amelie Forget, MSc, concluded that women who had an asthma flare-up in the first three months of pregnancy were 48 percent more likely to have a baby with at least one congenital defect than asthmatic mothers who did not have a flare-up in the first trimester.
The rate of birth defects among the children of mothers who experienced a flare-up was 12.8 percent, versus a rate of 8.9 percent for mothers with better-controlled asthma, according to study data.
In total, researchers analyzed more than 4,300 pregnancies through health care and pharmacy records.
The findings underscore the need to keep asthma well-managed throughout pregnancy, but especially in the first trimester - a crucial period for fetal development.
The American Academy of Allergy, Asthma & Immunology (AAAAI) recommends all pregnant women with a history of asthma consult with an allergist/immunologist to ensure the asthma is well-controlled.
When a pregnant woman has trouble breathing, as during an asthma attack, both mother and fetus can experience a drop in the level of oxygen in their blood. A fetus needs a consistent supply of blood for normal growth and survival.
Pregnant women, like all asthma patients, should avoid common asthma triggers such as house dust mites, animal dander and smoke, according to the AAAAI.
An allergist/immunologist can prescribe safe and effective medications for controlling asthma during pregnancy.
To learn more about asthma and pregnancy or to find an allergist/immunologist in your area, visit www.aaaai.org.
The Journal of Allergy and Clinical Immunology (JACI) is the official scientific journal of the AAAAI.
The AAAAI represents allergists, asthma specialists, clinical immunologists, allied health professionals and others with a special interest in the research and treatment of allergic disease. Established in 1943, the AAAAI has nearly 6,500 members in the United States, Canada and 60 other countries.

New reports in the January issue of **Annals of Allergy, Asthma & Immunology** include studies on:

Allergic disease linked to irritable bowel syndrome. See news release below. Original article: "Atopic irritable bowel syndrome: a novel subgroup of irritable bowel syndrome with allergic manifestations"- Mary C. Tobin, MD, Rush University Medical Center, Chicago; and others. Investigators found the likelihood of irritable bowel syndrome (IBS) was significantly higher in patients with seasonal allergic rhinitis (2.67 times), patients with allergic eczema (3.85 times), and patients with depression (2.56). Patients reporting symptoms of seasonal allergic rhinitis, allergic eczema, and asthma were 3.20 times more likely to fulfill the criteria for IBS.

New reports in the March issue of **Annals of Allergy, Asthma & Immunology** include studies on:

Nonallergists may under-diagnose or over-diagnose food allergy. Original Article: "Survey of Physicians´ Approach to Food Allergy, Part 2: Allergens, Diagnosis, Treatment, and Prevention" - Brian G. Wilson, M.D., Louisiana State University Health Sciences Center, Shreveport, La.; and others. Survey re-

sults indicate nonallergists differ markedly from allergists in diagnostic testing methods for food allergy - using more leukocytotoxic tests, specific IgE tests, and intradermal tests, but fewer percutaneous skin tests, specific IgE tests, and challenges. Allergists were more likely to rely on elimination of proven food allergens and less likely to use conventional elimination diets, rotation diets, and sublingual or subcutaneous hyposensitization. *This study was supported by the American College of Allergy, Asthma and Immunology.*

Asthma drugs often prescribed to children undiagnosed with asthma. Original article: "Asthma-related Medication Use Among Children in the United States" - James J. Korelitz, PhD, Westat, Rockville, Md.; and others. Retrospective analysis of medical and drug health care insurance claims of 4,259,103 children throughout the United States, aged birth through 17 years, shows 15 percent of all children were dispensed an asthma-related medication. Among children without an asthma diagnoses, 10 percent had a dispensed medication. Fifty-nine percent of children with an asthma diagnosis were dispensed an anti-inflammatory medication within 90 days after a claim. *Funding support was provided by the National Institutes of Health, National Institute of Child Health and Human Development.*

New reports in the May issue of **Annals of Allergy, Asthma & Immunology** include studies on:

Therapeutic alternatives for chronic urticaria. CME Review: "Therapeutic Alternatives for Chronic Urticaria: An Evidence-based Review, Part I" - Matt Morgan, M.D., University of Texas Southwestern, Dallas, and David A. Khan, M.D., Allergy, Asthma and Immunology of North Texas, McKinney, Texas. Investigators evaluate the use of alternative therapies for the common problem of chronic urticaria refractory to antihistamines, the first-line treatment, in an evidence-based manner performing MEDLINE searches. They conclude that alternative agents, including leukotriene modifiers, dapsone, sulfasalazine, hydroxychloroquine, colchicine, calcineurin inhibitors and mycophenolate, should be considered in patients with chronic urticaria who are both severely affected and unresponsive to antihistamines.

Inhaled corticosteroid not associated with reduction of endogenous cortisol. Original article: "Lack of Effect on Adult and Adolescent Hypothalamic-Pituitary-Adrenal Axis Function with use of Fluticasone Furoate Nasal Spray" - Deepen Patel, MD, Allied Research International Inc., Mississauga, Ontario, Canada; Paul Ratner, Sylvania Research Associates, San Antonio, Texas; and others. Authors indicate intranasal corticosteroids are considered to be the most effective treatment for allergic rhinitis (AR) when nasal congestion is the primary symptom. The newer intranasal corticosteroids, such as fluticasone propionate, mometasone furoate, and now fluticasone furoate, are sprayed directly to the inferior turbinate, and subsequently, there is a topical effect rather than a systemic one. This study was funded by GlaxoSmithKline R&D Ltd and was designed by the funding source in consultation with the principal investigators. Statistical analyses were performed by the funding source. The principal investigators conducted the study and participated in the interpretation and reporting of the study and in the decision to submit the article for publication in conjunction with the funding source.

American Academy of Allergy Asthma & Immunology (AAAAI) - For immediate release, June 19, 2008

Food-allergic children with asthma may require extra emergency medication

MILWAUKEE - New research findings suggest that some food-allergic children may not be equipped with enough potentially life-saving medication to reverse a severe allergic reaction.

According to research to be published in an upcoming issue of the *Journal of Allergy and Clinical Immunology*, a second dose of epinephrine - the drug of choice for treating severe allergic reactions - was needed in nearly 1-of-5 cases of food-induced anaphylaxis in children.

Nearly all patients who required multiple doses of epinephrine also suffered from asthma.

Though further studies are needed, these findings point to asthma as a risk factor for severe anaphylaxis and could influence how epinephrine is prescribed to children.

Many children and adults at risk of severe allergic reactions are currently advised to carry only a single epinephrine auto-injector, which is administered when a severe reaction occurs.

In her study article, lead author Kirsi M. Järvinen, MD, PhD, writes that "the recommendation to carry two doses of epinephrine should as minimum be extended to individuals with asthma and significant food allergies."

Järvinen and colleagues from Mount Sinai School of Medicine studied the histories of 413 food-allergic children. They identified 78 patients who had received epinephrine to treat a total of 95 anaphylactic reactions. Parents of the children were asked to recall the suspected food trigger, how rapidly symptoms developed and the timing of treatment.

Of the 95 reactions treated with epinephrine, a second dose of the medication was administered in 19 percent of cases (18 patients). A third dose was required in 6 percent of cases (6 cases). Of those who received multiple doses, all but one (94 percent) were also diagnosed with asthma.

In a surprise finding, the survey results also indicated that many children did not receive epinephrine, despite past severe reactions. While 51 percent of total patients studied reported a past history of anaphylactic symptoms, only 20 percent had ever used epinephrine.

Anaphylaxis is a serious, potentially life-threatening allergic reaction that can affect the cardiovascular, respiratory or gastrointestinal systems of the body. An estimated 100-150 people in the United States die each year from anaphylaxis, according to the American Academy of Allergy, Asthma & Immunology (AAAAI). Anaphylaxis is most commonly caused by allergic reactions to food, insect stings and medication.

Food allergies affect 3 million American children, including 1 in 17 children under the age of 3, according to the AAAAI. Additionally, about 9million children in the United States have asthma.

The *Journal of Allergy and Clinical Immunology* is the official scientific journal of the AAAAI.

American Academy of Allergy Asthma & Immunology (AAAAI) - For immediate release, July 15, 2008

Children may build tolerance to egg allergy

MILWAUKEE – New research suggests that beating childhood egg allergy is literally a piece of cake.

In a preliminary study, researchers in Greece demonstrated that gradually exposing allergic children to heat-treated egg – such as those in baked goods – could help them overcome the allergy.

In the trial, 94 children were regularly given tiny amounts of cake containing egg. Over a period of several months, the quantity of cake was gradually increased. Eighty-seven children (90 percent) were able to eat the maximum amount without symptoms.

After six months of daily doses of the processed egg, those children were challenged to eat egg that was not cooked to the same degree. More than 95 percent had no reaction and were believed to have outgrown the allergy.

The findings will be published in an upcoming issue of the *Journal of Allergy and Clinical Immunology*, the official scientific journal of the American Academy of Allergy, Asthma & Immunology (AAAAI).

According to the AAAAI, one in 17 children under age 3 suffers from a food allergy. Hen's egg is among the most common allergens for children. And while many children with egg allergy outgrow the sensitivity by the time they enter school, until then eliminating all egg from the diet is the only effective management.

This study suggests that deliberate exposure to heat-treated egg may speed that tolerance.

The AAAAI represents allergists, asthma specialists, clinical immunologists, allied health professionals and others with a special interest in the research and treatment of allergic disease. Established in 1943, the AAAAI has nearly 6,500 members in the United States, Canada and 60 other countries. The AAAAI promotes public education of allergy and asthma through its Web site, www.aaaai.org.

American Academy of Allergy Asthma & Immunology (AAAAI) - For immediate release, July 19, 2008

Antidote for chemotherapy allergy allows cancer patients to receive medicine safely

MILWAUKEE – New research findings may be the cure for cancer patients who have developed allergies to chemotherapy treatment.

In a study to be published in an upcoming issue of the Journal of Allergy and Clinical Immunology, Mariana C. Castells, MD, Phd, and colleagues at Brigham Women's Hospital and the Dana-Farber Cancer Institute in Boston unveil new data demonstrating the success and safety of rapid desensitization.

The treatment allows nearly all patients to temporarily tolerate the chemotherapy drugs to which they previously experienced allergic reactions.

The standardized procedure takes four to eight hours and involves administering the targeted dose of medicine to patients in incremental steps.

Of 98 patients tested during a total 413 desensitization treatments, 94 percent had no reaction or only mild reactions from the medication. No life-threatening reactions or deaths occurred during the study.

Patients studied were primarily women receiving treatment for breast, ovarian or other gynecologic cancers.

During the study, patients were successfully desensitized to seven common chemotherapy drugs: carboplatin, cisplatin, oxaliplatin, paclitaxel, liposomal doxorubicin, doxorubicin and rituximab.

Previous studies have indicated that up to 27 percent of patients receiving more than seven cycles of some common chemotherapy drugs develop allergic sensitivity to the medication. With some drugs, many patients experience a reaction on their first exposure. The reactions can be severe and potentially fatal.

This presents healthcare professionals and patients with a paradoxical challenge: continue potentially life-saving treatment while risking a deadly allergic reaction or avoid the life-threatening reaction by switching to a less-effective drug?

This new research offers a third option. Brigham Women's Hospital has already adopted the standardized rapid desensitization. Researchers hope other institutions will follow suit.

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