

Discrepancy between sensitization to inhaled allergens and respiratory symptoms in pediatric patients with type 1 diabetes mellitus

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According to the 'Th₁/Th₂ paradigm', children with type 1 diabetes mellitus (T1DM) should have a lower risk of developing allergic sensitization and, because of the involvement of insulin in modulating airway inflammation, different frequency or severity in allergy-related respiratory manifestations. This article aims at evaluating the frequency and type of allergic sensitization and its respiratory manifestation, asthma and/or rhinitis, in a group of pediatric patients with T1DM. Patients (112) with T1DM, 7.8–16.9 yr of age (63 males and 49 females) were evaluated. Skin prick test (SPT) reactivity to the most common classes of aeroallergens were performed and compared with data obtained in 709 school-aged children. The frequency of sensitization was not different in the T1DM and in the control subjects (43.7% and 40.8%, respectively; $p = 0.55$), with similar proportions of individuals sensitized to one allergen (32.7% and 38.1%, respectively; $p = 0.47$). In both groups, sensitization to house dust mite allergens was the most frequently detected (69.4% and 65.4%, respectively; $p = 0.59$), with a higher proportions of individuals sensitized to *Graminae* (+ *Cynodon dactylon*; $p < 0.0001$) and a lower, but weakly significant, proportion sensitized to *Parietaria* ($p = 0.03$) in the T1DM group, as compared with controls. No differences were found between T1DM and control groups in the proportion of individuals reporting rhinitis (26.8% and 29.2%; $p = 0.60$). However, comparing separately sensitized and non-sensitized subjects, a lower proportion of rhinitis subjects was detected in the non-sensitized T1DM patients, when compared with the non-sensitized control subjects ($p = 0.01$). In addition, no differences were detected between T1DM and control groups in frequency of symptoms related to 'lifetime asthma', i.e., asthma episodes during life (14.3% and 16.5%, respectively; $p = 0.55$), also when sensitized and non-sensitized subjects were evaluated separately ($p = 0.12$ and $p = 1.00$, respectively). However, no T1DM patient had 'actual asthma', i.e., asthma episodes in the last year, vs. 5.8% of the individuals in the control group ($p = 0.009$), the difference being mostly ascribed to sensitized subjects ($p = 0.012$). Finally, out of the 16 T1DM patients with 'lifetime asthma', 15 had mild intermittent disease and only one mild persistent disease. T1DM does not seem to play a downregulating role on the development of allergic sensitization to aeroallergens, but may lower the frequency or the severity of its clinical manifestations at respiratory level.

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The regulation of the immune response involves two subtypes of T-lymphocytes with helper function (Th): (i) the Th₁ cells, which produce cytokines, such as IFN- γ and IL-2, known to be important in the defense against infections and tumors, but also in the development of organ-specific autoimmune disorders and (ii) the Th₂ cells, producing cytokines such as IL-4, IL-5, IL-9, and IL-13, which are involved in the protection against parasites but also promote the development of IgE-mediated atopic sensitization (1, 2).

Different studies have been performed to evaluate whether autoimmune diseases (Th₁-related) and allergic diseases (Th₂-related) are mutually exclusive and unlikely to be present in the same individual (2–4). This concept is based on the 'Th₁/Th₂ paradigm', so that a reduction of the Th₁ compartment should enhance a proliferation or expansion of Th₂ clones and vice versa (4).

A Th₁ protective role against the development of allergic symptoms by reducing Th₂ immunological reaction has been suggested, but reports on the interaction between Th₁-related autoimmune diseases, such as type 1 diabetes mellitus (T1DM) and Th₂-related atopic sensitization and/or its clinical manifestations are conflicting. Indeed, while there are few reports showing an increased incidence of asthma in patients with autoimmune diseases (5) or no evidence of an inverse relationship between Th₂-mediated atopy and Th₁-mediated autoimmune disorders (6, 7), reduction in the frequency of allergic symptoms in children with T1DM has been reported in different patient populations (6, 8, 9). Interestingly, only in one study IgE sensitization was evaluated and no difference in the proportion of skin prick test positive subjects between T1DM children and controls was found, despite the lower frequency of allergic symptoms (wheezing, asthma and allergic rhinitis) in the T1DM group (6).

This finding suggests that factors related to diabetes mellitus, possibly not related to the Th₁ and Th₂ balance, may decrease the clinical expression of the respiratory manifestations in children with T1DM. With this background, a study was designed to evaluate the frequency and type of allergic sensitization and its respiratory manifestations, asthma and/or rhinitis, in a group of children with T1DM, when compared with school-aged children from the general population.

Materials and methods

Study population

Children with T1DM were consequently enrolled in the patients group at the Regional Childhood

Diabetes Centre of the G. Gaslini Institute, from July 1, 2005 to February 17, 2006. There were 63 males and 49 females, aged between 7.8 and 16.9 yr (mean age: 12.8 yr). The Institutional Ethical Committee of the G. Gaslini Institute approved the protocol. Signed informed parental consent and the child's assent (if the child was ≥ 10 yr old) were obtained. Diabetes diagnosis was based on validated criteria (10) (i) positive pancreatic β -cells autoantibodies; (ii) signs and symptoms of diabetes mellitus; and (iii) insulin-dependence. Seven hundred and nine school-aged children living in Liguria were evaluated, as control population, whose demographic and clinical characteristics have been previously described (11).

Data collection

Information on demography, symptoms of wheeze, asthma during the postnatal period and at the time of the survey were collected in cases and controls. A slight modified version of the questionnaire investigating the frequency of allergic respiratory symptoms, from the International Study of Asthma and Allergies in Childhood (ISAAC) (12), was administered to parents of all children enrolled in the study and the demographic and clinical data were collected by physicians (EV, MAT) involved in the study. The following respiratory symptoms were considered: (i) rhinitis symptoms (itchy and/or runny and/or blocked nose, not associated with a cold or a flu) and (ii) asthma-related symptoms (dyspnea, wheezing, recurrent dry cough or effort-related symptoms). The severity of asthma was classified with modification to the Global Initiative for Asthma (GINA) guidelines according to the children's frequency of symptoms (13), while asthma episodes during life were classified as 'lifetime asthma' and asthma episode in the last year as 'actual asthma'.

Skin prick test

Skin prick tests were performed according to the methodology and criteria proposed by the European Academy of Allergology and Clinical Immunology (EAACI) (14). The test uses a panel of allergens extracts, histamine solution (10 mg/ml) as positive control and glycerol buffer diluent of allergenic preparations as negative control. Wheals at least 3 $\times$ 3 mm greater than wheals at the site of negative control are regarded as positive (14).

In this study, the most frequent aeroallergens in our geographic area were tested (Lofarma, Milan, Italy): house dust mites (*Dermatophago-*

ides pteronyssinus and *Dermatophagoides farinae*); pollens (*Parietaria Officinalis*; *Graminae* mix; *Cynodon dactylon*; *Compositae*; *Betulaceae*: Birch and Hazel-nut tree; *Oleaceae*: Olive tree); pet allergens (cat or dog epithelium); moulds (*Aspergillus*, *Alternaria alternata*) (15).

Patients with T1DM were considered mono-sensitized when the SPT was positive for one allergen class (i.e., mites, pollens, pets or moulds) and poly-sensitized when the SPT was positive for two or more allergen classes.

Spirometry

Spirometry was performed in T1DM patients according the guidelines provided by the *American Thoracic Society* (16). All the children were able to obtain at least three technically acceptable breathing maneuvers with the spirometer (MedGraphics, St. Paul, MN USA). The results were compared with reference values obtained from a well-defined population, identified by the *American Thoracic Society*, of healthy subjects comparable for gender, height, weight and then expressed in percentage (16).

Statistical methods

Descriptive statistics were firstly performed and qualitative data (gender, positive result to prick test, etc.) were reported in terms of absolute frequencies and percentages; means and standard deviations (s.d.), or medians and minimum and maximum values (min, max) were reported in case of quantitative variables (FVC, FEV₁, etc.).

As a second step, a bivariate analysis was performed; comparison of qualitative data (i.e., gender, allergic sensitization data) between cases and controls was made by the chi-square test or by the Fisher's Exact test whenever expected frequencies were less than five. Comparison of quantitative variables between the two groups of subjects was made by means of the Student's *t*-test or the Mann-Whitney *U*-test whenever the homoscedasticity assumption was not fulfilled or when the distribution of the variable was clearly skewed. All tests were two sided and a *p*-value <0.05 was considered statistically significant. The statistical package used were the 'Statistica release 6' (StatSoft Corp., Tulsa, OK, USA) for all the analyses.

Results

Clinical and demographic features of T1DM patients and of control group

The demographic and clinical characteristics of the 112 patients with T1DM recruited are

Table 1. Baseline data and characteristics of the patients with type 1 diabetes mellitus

	N	Mean (s.d.)	Median	Min	Max
Age at the visit (yr)	112	12.8 (2.5)	13.1	7.8	16.9
Age at the onset of type 1 diabetes (yr)	112	7.3 (3.4)	7.1	1.1	14.8
Disease duration (yr)	112	5.5 (3.6)	5.3	0.3	14.7
BMI (kg/m ²)	112	19.4 (3.2)	18.7	13.7	31.3
Insulin dose (U/kg/day)	112	0.85 (0.24)	0.90	0.04	1.50
Hb A1 C levels (%)	112	8.4 (1.1)	8.2	5.9	12.6

s.d., standard deviation; Min–Max, minimum and maximum values; BMI, body mass index.

Hb A1 C levels (%), glycosilated hemoglobin.

reported in Table 1. A subgroup of T1DM patients suffered of a concomitant autoimmune disease: 14 patients had celiac disease, 12 Hashimoto's thyroiditis and one patient both celiac disease and Hashimoto's thyroiditis. Subjects in the control group were comparable in relation to gender distribution (56.3% males among the cases and 53.0% among the controls; chi-square test; *p* = 0.53) and in relation to age (12.8 and 12.4 yr mean age of the cases and of the controls, respectively).

Frequency and type of allergic sensitization

The frequency of allergic sensitization in cases and controls is shown in Table 2. The frequency of sensitization was not different in the T1DM and in the control subjects (43.7% and 40.8%, respectively; *p* = 0.55), with similar proportions of individuals sensitized to one allergen (32.7% and 38.1%, respectively; *p* = 0.47).

In both groups, the most frequent type of sensitization detected was to house dust mite allergens (69.4% and 65.4%, respectively;

Table 2. Specific allergic sensitizations in cases and controls

	Sensitized patients with T1DM (N = 49) 43.7%	Sensitized controls (N = 289) 40.8%	p-value (0.55)
House dust mites	34 (69.4)	189 (65.4)	0.59
<i>Parietaria</i>	11 (22.4)	112 (38.8)	0.03
<i>Graminae</i> (+ <i>Cynodon dactylon</i>)	34 (69.4)	103 (35.6)	<0.0001
<i>Compositae</i> (+ <i>Absinthium</i>)	3 (6.1)	37 (12.8)	0.18
Birch or Hazelnut tree	10 (20.4)	49 (17.0)	0.56
Olive tree	15 (30.6)	54 (18.7)	0.055
Cat or dog epithelium	10 (20.4)	44 (15.2)	0.36
<i>Alternaria</i>	4 (8.2)	21 (7.3)	0.77†
<i>Aspergillus</i>	2 (4.1)	16 (5.5)	1.00†

Values are expressed as N (%).

All *p*-values refer to the chi-square test unless otherwise specified.

†Fisher's Exact test.

Table 3. Allergic mono-sensitizations in cases and controls

	No. mono-sensitized/total no. sensitized to specific allergens (%)		p-value
	Patients with T1DM	Controls	
House dust mites	12/34 (35.3)	58/189 (30.7)	0.59
<i>Parietaria</i>	0/11 (0)	16/112 (14.3)	0.36†
<i>Graminae</i> (+ <i>Cynodon dactylon</i>)	3/34 (8.8)	15/103 (14.6)	0.56†
<i>Compositae</i> (+ <i>Absinthium</i>)	0/3 (0)	2/37 (5.4)	1.00†
Birch o Hazelnut tree	0/10 (0)	2/49 (4.1)	1.00†
Olive tree	1/15 (6.7)	5/54 (9.3)	1.00†
Cat or dog epithelium	0/10 (0)	8/44 (18.2)	0.33†
<i>Alternaria</i>	0/4 (0)	3/21 (14.3)	1.00†
<i>Aspergillus</i>	0/2 (0)	2/16 (12.5)	1.00†

Values are expressed as N (%).

All p-values refer to the chi-square test unless otherwise specified.

†Fisher's Exact test.

p = 0.59), with a higher proportions of individuals sensitized to *Graminae* (+ *Cynodon dactylon*; p < 0.0001) and a lower, but weakly significant, proportion sensitized to *Parietaria* (p = 0.03) in the T1DM group, as compared with controls. Finally, evaluation of the distribution of the mono-sensitizations in cases and controls was similar in the two groups (p = NS, each comparison; Table 3).

The frequency and type of sensitization was not different in the subgroup of T1DM patients with concomitant celiac disease or Hashimoto's thyroiditis, when compared with the whole T1DM group and the control group. Indeed, 6/14 (42.9%) children with celiac disease and 6/12 (50.0%) with Hashimoto's thyroiditis were sensitized to at least one allergen (p = NS, each comparison). Similarly to the whole T1DM group and the control group, specific sensitizations to house dust mites and pet allergens were equally distributed among the two subgroups with frequencies similar to those observed in the whole T1DM patient group and in the control group (p = NS, each comparison) (not shown).

Frequency and type of respiratory manifestations

Evaluating the frequency of respiratory manifestations, no differences were found between T1DM and control groups in the proportion of individuals reporting rhinitis (26.8% and 29.2%; p = 0.60). However, comparing separately sensitized and non-sensitized subjects, a lower proportion of rhinitis subjects was detected in the non-sensitized T1DM patients, when compared with the non-sensitized control subjects (p = 0.01). In addition, no differences were

Table 4. Respiratory symptoms in cases and controls

	Patients with T1DM	Controls	p-value*
Rhinitis	30/112 (26.8)	207/709 (29.2)	0.60
Rhinitis (in sensitized subjects)	25/49 (51.0)	117/289 (40.5)	0.17
Rhinitis (in not sensitized subjects)	5/63 (7.9)	90/420 (21.4)	0.01
'Lifetime' asthma	16/112 (14.3)	117/709 (16.5)	0.55
'Lifetime' asthma (in sensitized subjects)	10/49 (20.4)	77/289 (26.6)	0.36
'Lifetime' asthma (in not sensitized subjects)	6/63 (9.5)	40/420 (9.5)	1.00
'Actual' Asthma	0/112 (0.0)	41/709 (5.8)	0.009
'Actual' Asthma (in sensitized subjects)	0/49 (0.0)	30/289 (10.4)	0.012†
'Actual' Asthma (in not sensitized subjects)	0/63 (0.0)	11/420 (2.6)	0.37†

Values are expressed as N (%).

*All p values refer to the chi-square test unless otherwise specified.

†Fisher's Exact test.

found between T1DM and control groups in the proportion of individuals reporting 'lifetime asthma' (14.3% and 16.5%, respectively; p = 0.55), also when sensitized and non-sensitized subjects were evaluated separately (p = 0.12 and p = 1.00, respectively). In contrast, no T1DM patient had 'actual asthma', i.e., referred asthma episodes in the last year, vs. the 5.8% of the individuals in the control group (p = 0.009), the difference being mostly ascribed to sensitized subjects (p = 0.012) (Table 4).

Finally, the proportion of individuals reporting respiratory manifestations was similar in the two T1DM subgroups with concomitant celiac disease or Hashimoto's thyroiditis, being 21.4% and 25.0%, respectively, and similar to that observed in the whole T1DM patient group and in the control group (p = NS, each comparison).

Asthma severity in T1DM patients

Asthma was assessed according to the patient's frequency of symptoms together with lung function. Of 16 T1DM patients with 'lifetime asthma', 15 had mild intermittent disease and one patient, sensitized to house dust mites, mild persistent disease. All T1DM mild intermittent asthmatic patients had normal lung function, with FEV₁ ≥ 80% predicted and a FEV₁/FVC ratio ≥ 71, while the persistent asthma patient, showed a mild spirometric impairment (FEV₁ < 79% predicted and a FEV₁/FVC ratio < 69%). The presence of nocturnal symptoms was never reported. Fifty-four percent of the children used medications for respiratory symptoms during life on 'as-needed' basis, and none, including the

patient with mild persistent asthma, used continuously inhaled corticosteroid or other 'controller medications' (cromones, long-acting inhaled β_2 -agonists, leukotriene modifiers). Antihistamines was the most common treatment used on 'as necessary' basis (45.4%) to control allergic rhinitis. No T1DM patients used anti-asthmatic medications during the year preceding the enrolment.

Discussion

Evaluating children and adolescents with T1DM, we found that the frequency of sensitization to at least one allergen class was not different in the T1DM and in the control subjects, with similar proportions of mono- and polysensitized individuals. In addition, no differences were detected between T1DM and control groups on frequency of asthma symptoms during life, also when only sensitized individuals were evaluated, but T1DM patients did not show asthma exacerbations in the course of the last year, in sensitized and not sensitized patient.

The lack of difference in the frequency of skin prick test positivity and of respiratory manifestations between the two study population, including the subgroups of children with T1DM and concomitant celiac disease or Hashimoto's thyroiditis, shown in this study is in agreement with previous findings (7, 9, 17, 18) and speaks against the customary categorization of Th₁/Th₂ diseases. These results do not invalidate the hypothesis that Th₁/Th₂ balance may play an important role in the pathogenesis of asthma and T1DM. However, Th₂-type cytokine expression has recently been identified as possible contributors to the pathogenesis of autoimmune diseases (19, 20), while *in vitro* stimulation of peripheral blood mononuclear cells with disease-specific recombinant antigens demonstrated a similar Th₁ and Th₂ secretory pattern in patients with T1DM and asthma, suggesting a unique Th₁/Th₂ balance (21). A similar study also demonstrated that peripheral blood mononuclear cells from children suffering from both diabetes and asthma may display a unique cytokine secretion pattern, distinct from those of solely diabetic, asthmatic and healthy children, but suggested that those with both diseases may have a defect in regulation of IL-10 (22). It is clear that other T-cell subsets (e.g., T regulatory cells) may be involved in the pathogenesis of autoimmune and allergic disorders and that the Th₁/Th₂ paradigm might be an oversimplification (2). In a previous study at T1DM onset, we observed a significantly lower percentage of peripheral CD4⁺ and CD8⁺ cells

producing IFN- γ in patients compared with controls and subjects at risk. The 15-month follow-up patients showed significantly lower percentage of CD4⁺ and CD8⁺ cells producing IFN- γ compared to the other groups: the absence of IFN- γ production makes β -cells highly susceptible to viral infection, enhancing autoimmune disease (23). Finally, the observation of strong positive associations between the occurrence of TDM1 and asthma in the general population also suggests that factors influencing the susceptibility to these disorders may include genetic predisposition, early environmental exposures, or both (17, 18). The differences in type of sensitization found in our study population, i.e., higher percentage of sensitization to *Graminae* and lower to *Parietaria* detected in the T1DM group when compared with controls, have no apparent explanation and must probably be confirmed in a larger population.

Independently from the allergic sensitization, a reduction in the frequency of allergic respiratory symptoms in children with T1DM, evaluated by written questionnaire, has been reported in different patient populations (6, 8, 9) and this observation has been explained by a variety of factors, related to the disease or to its treatment.

In contrast to what seen in asthma, patients with diabetes mellitus seem to have a tendency to show or develop decreased airway reactivity to a variety of stimuli. Evaluating patients with T1DM it was shown that not only they were less affected by asthma than control subjects, although their positive family history of atopy, but also that they had a lower bronchial reactivity to carbachol inhalation challenge (24). Since the duration of disease in T1DM patients was positively correlated with the results of carbachol testing, it was suggested that reduced bronchial reactivity might be an indication of initial damage to the autonomic nervous system (24). Moreover, diabetic patients with autonomic neuropathy, demonstrate a decrease response in vagal reflex-induced bronchoconstriction, as they did not show a significant fall in specific airways conductance in response to eucapnic hyperventilation, a reaction which is thought to be largely mediated via neural mechanisms (25).

These findings are supported by animal models of diabetes in which a reduction in sensory neuropeptide release (26) and an increased lung M2 muscarinic receptors function, leading to a decreased vagally induced bronchoconstriction, has been demonstrated (27). On the contrary, it was shown that, in diabetic animals, insulin restores M2 receptor function (and therefore the vagally mediated hyper-reactivity) (27), reverse

the downregulation of eosinophil accumulation in the airways following allergen challenge (28), and the reduced bronchial contraction associated with inhibition of mast cell degranulation and histamine release (29).

These data may suggest that also in humans insulin could be required for development of airway inflammation, including eosinophil accumulation and mast cell activation, in addition to loss of neuronal M2 muscarinic receptor function and subsequent hyper-responsiveness in allergic asthma. We should therefore expect a 'slightly reduced' or 'normal' expression of respiratory symptoms in T1DM patients, treated by definition with insulin.

Indeed, in this study no differences were found between T1DM and control groups on the proportion of individuals reporting 'lifetime asthma' symptoms, i.e., including the period of life when T1DM was not affecting the subject. In contrast, there were no cases of 'actual asthma', suggesting that the occurrence of asthma symptoms during T1DM could be rare and mild, also in allergic subjects. The finding of a lower proportion of subjects with symptoms of rhinitis in the not sensitized T1DM subgroups, is also in favor of the hypothesis that diabetes mellitus could to be associated with a lower airway reactivity to a variety of stimuli and that insulin could be required for mast cell activation (24, 29).

We do not have data on the severity of asthma in our control group. However, although the proportion of children with asthma in T1DM patients is not different from that reported in a similar studies performed in a general population of school-aged children (30), it is interesting that only one of our diabetic patients has had mild persistent disease and none moderate or severe disease and that none used anti-asthma medications on a regular basis, but overall that in the last year none of T1DM allergic patients have asthma exacerbation or a mild intermittent disease.

Finally, the hypothesis of a reduced clinical expression of allergic sensitizations in diabetes is also supported by a recent preliminary report showing that in a group of children and adolescents with TDM1 the presence of latex sensitization was not apparently accompanied by an increased risk of latex anaphylaxis (31).

In conclusion, the presence of T1DM does not seem to have a protective role against the development of allergic sensitization to aero-allergens, but it could influence the frequency and/or severity of its clinical manifestations at respiratory level in the last year of evaluation. The hypothesis that T1DM children and adolescents may not have symptoms of asthma,

because of reduction in sensory neuropeptide release, increased function of neuronal inhibitory M2 muscarinic receptors and decreased accumulation and activation of inflammatory cells in the airways should, however, be supported by longitudinally and specifically designed studies.

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References

1. MOSMANN T, CHERWINSKI H, BOND M, GIEDLIN M, COFFMAN R. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol* 1986; 136: 2348–57.
2. MOSMANN T, SAD S. The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunol Today* 1996; 17: 138–46.
3. BERGER A. Th1 and Th2 responses: what are they? *BMJ* 2000; 321: 424.
4. SINGH V, MEHROTRA S, AGARWAL S. The paradigm of Th1 and Th2 cytokines: its relevance to autoimmunity and allergy. *Immunol Res* 1999; 20: 147–61.
5. SIMPSON C, ANDERSON W, HELMS P, et al. Coincidence of immune-mediated diseases driven by Th1 and Th2 subsets suggests a common aetiology. A population-based study using computerized general practice data. *Clin Exp Allergy* 2002; 32: 37–42.
6. SHEIKH A, SMEETH L, HUBBARD R. There is no evidence of an inverse relationship between TH2-mediated atopy and TH1-mediated autoimmune disorders: lack of support for the hygiene hypothesis. *J Allergy Clin Immunol* 2003; 111: 131–5.
7. STRÖMBERG L, LUDVIGSSON G, BJÖRKSTÉN B. Atopic allergy and delayed hypersensitivity in children with diabetes. *J Allergy Clin Immunol* 1995; 96: 188–92.
8. TZENG S, HSU S, FU L, CHI C. Prevalence of atopy in children with type 1 diabetes mellitus in central Taiwan. *J Microbiol Immunol Infect* 2007; 40: 74–8.
9. CAFFARELLI C, CAVAGNI G, PIERDOMENICO R, CHIARI G, SPATTINI A, VANELLI M. Coexistence of IgE-mediated allergy and type 1 diabetes in childhood. *Int Arch Allergy Immunol* 2004; 134: 288–94.
10. GENUTH S, ALBERTI K, BENNETT P, et al. Follow-up report on the diagnosis of diabetes mellitus. *Diabetes Care* 2003; 26: 3160–7.
11. CRIMI P, MINALE P, TAZZER C, ZANARDI S, CIPRANDI G. Asthma and rhinitis in schoolchildren: the impact of allergic sensitization to aeroallergens. *J Investig Allergol Clin Immunol* 2001; 11: 103–6.
12. ASHER M, KEIL U, ANDERSON H, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J* 1995; 8: 483–91.
13. GLOBAL STRATEGY FOR ASTHMA MANAGEMENT AND PREVENTION, Global Initiative for Asthma (GINA) 2007. Available from: <http://www.ginasthma.org>.
14. THE EUROPEAN ACADEMY OF ALLERGOLOGY AND CLINICAL IMMUNOLOGY. Position paper: Allergen standardization and skin tests. *Allergy* 1993; 48: 48–82.
15. FASCE L, TOSCA M, SILVESTRI M, OLCESE R, PISTORIO A, ROSSI G. 'Early' cat ownership and the risk of sensitization and allergic rhinitis in Ligurian children

- with respiratory symptoms. *Ann Allergy Asthma Immunol* 2005; 94: 561–5.
16. AMERICAN THORACIC SOCIETY. Standardization of spirometry, 1994 update. *Am J Respir Crit Care Med* 1995; 152: 1107–36.
17. STENE L, NAFSTAD P. Relation between occurrence of type 1 diabetes and asthma. *Lancet* 2001; 357: 607–8.
18. KERO J, GISSLER M, HEMMINKI E, ISOLAURI E. Could TH1 and TH2 diseases coexist? Evaluation of asthma incidence in children with coeliac disease, type 1 diabetes, or rheumatoid arthritis: a register study *J Allergy Clin Immunol* 2001; 108: 781–3.
19. HESSE M, BAYRAK S, MITCHISON A. Protective major histocompatibility complex genes and the role of interleukin-4 in collagen-induced arthritis. *Eur J Immunol* 1996; 26: 3234–7.
20. BALASA B, SARVETNICK N. Is pathogenic humoral autoimmunity a Th1 response? Lessons from (for) myasthenia gravis *Immunol Today* 2000; 21: 19–23.
21. RACHMIEL M, BLOCH O, BISTRITZER T, et al. TH1/TH2 cytokine balance in patients with both type 1 diabetes mellitus and asthma. *Cytokine* 2006; 34: 170–6.
22. KAINONEN E, RAUTAVA S, KORKEAMÄKI M, ISOLAURI E. Unique cytokine secretion profile in children with both type I diabetes and asthma distinct from that of solely diabetic or asthmatic children. *Cytokine* 2006; 34: 198–205.
23. AVANZINI M, CIARDELLI L, LENTA E, et al. IFN-gamma low production capacity in type 1 diabetes mellitus patients at onset of disease. *Exp Clin Endocrinol Diabetes* 2005; 113: 313–7.
24. VILLA M, CACCIARI E, BERNARDI F, CICOGNANI A, SALARDI S, ZAPULLA F. Bronchial reactivity in diabetic patients. Relationship to duration of diabetes and degree of glycemic control. *Am J Dis Child* 1988; 142: 726–9.
25. HEATON R, GUY R, GRAY B, WATKINS P, COSTELLO J. Diminished bronchial reactivity to cold air in diabetic patients with autonomic neuropathy. *Br Med J (Clin Res Ed)* 1984; 289: 149–51.
26. SZILVÁSSY J, SZIKLAI I, HORVATH P, et al. Feeble bronchomotor responses in diabetic rats in association with decreased sensory neuropeptide release. *Am J Physiol Lung Cell Mol Physiol* 2002; 282: L1023–30.
27. BELMONTE K, JACOBY D, FRYER A. Increased function of inhibitory neuronal M2 muscarinic receptors in diabetic rat lungs. *Br J Pharmacol* 1997; 121: 1287–94.
28. BELMONTE K, FRYER A, COSTELLO R. Role of insulin in antigen-induced airway eosinophilia and neuronal M2 muscarinic receptor dysfunction. *J Appl Physiol* 1998; 85: 1708–18.
29. CAVALHER-MACHADO S, DE LIMA W, DAMAZO A, et al. Down-regulation of mast cell activation and airway reactivity in diabetic rats: role of insulin. *Eur Respir J* 2004; 24: 552–8.
30. KO F, WANG H, WONG G, et al. Wheezing in Chinese schoolchildren: disease severity distribution and management practices, a community-based study in Hong Kong and Guangzhou. *Clin Exp Allergy* 2005; 35: 1449–56.
31. BOSTANCY I, DALLAR Y, UNSAL SAC R, KARAKOC E, DOGANAY B. Latex allergy risk assessment in children and adolescents with type I diabetes mellitus. *Pediatr Allergy Immunol* 2007; 18: 687–91.