

# Significant decrease of IgE antibodies after a three-year controlled study of specific immunotherapy to pollen allergens in children with allergic asthma

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**Abstract.** – **Background:** Specific immunotherapy (SIT) in children being not an optional treatment should be administered as soon as possible, also in children aged 2-3 years, due to the very early asthma and rhinitis onset, contrarily to opponents continuing to stress the danger of anaphylactic reactions without displaying reliable data.

**Materials and Methods:** We report 56 children who underwent SIT and 56 controls seen consecutively at the Allergy and Immunology Division, Department of Pediatrics, University of Rome "La Sapienza". The control group was treated with all appropriate medications.

**Results:** They were highly in favor of SIT with statistically significant differences. We stress that IgE antibodies significantly decreased after treatment only in the study group, and IgG antibodies very significantly increased after treatment only in the study group.

**Discussion:** We demonstrate that SIT is the only treatment which can alter the natural course of respiratory diseases, whereas drugs represent only a symptomatic treatment.

**Key Words:**

Pediatric SIT, Pediatric asthma, Allergic rhinitis, Very early onset, IgE and IgG antibodies.

## Introduction

Specific immunotherapy (SIT) has been the cornerstone of the treatment of allergic diseases for nearly a century and has been accepted worldwide into the therapeutic armamentarium of doctors interested in the management of children with respiratory allergies, including allergic rhinitis (AR) and asthma<sup>1-14</sup>.

Several mechanisms of SIT efficacy can be summarized as follows<sup>15</sup>:

### A) Reduction of IgE production

- 1) decrease in seasonal IgE boost
- 2) long-term reduction of antigen-specific IgE

### B) Increment of Antigen-specific IgG

- 1) increases in IgG4
- 2) correlation of SIT efficacy with increases of specific IgG competing with IgE on airway mediator cells

### C) Induction of Specific T Suppressor Cells

### D) Production of anti-idiotypic antibodies

### E) Diminished Mediator Release from Sensitized Cells

- 1) reduction of human blood basophils reactivity to allergens
- 2) reduced production of chemotactic factors for eosinophils
- 3) reduction of airway eosinophil accumulation

### F) Diminution of Tissue Responsiveness

- 1) reduced skin late phase response to specific allergens
- 2) reduced bronchial hyperreactivity
- 3) reduced nasal responses to mediators

### G) Immunologic Unresponsiveness

- 1) reduced human T lymphocyte proliferation and cytokine synthesis in response to allergens
- 2) potential switching from Th2 to Th1
- 3) reduced expression of CD23 on peripheral B cells<sup>15-17</sup>.

Systemic reactions to SIT have been reported since 1911, when this therapy was introduced into clinical practice<sup>18</sup>. The preva-

lence of systemic reactions has been estimated in adults, whereas at present there are a few data in children<sup>6,13,18,19</sup>. According to these studies performed on a small number of children with asthma, systemic reaction rate was actually zero using a mite extract<sup>13</sup>, between 80 to 100% using a highly purified and standardized mold extract<sup>6</sup>, whereas a case of anaphylactic shock occurred out of 1,056 children (0.09%), within a few seconds following the injection of the maintenance dose of the mite extract<sup>19</sup>, and in a subsequent study of 300 children only 5 children (1.6%) presented 5 unwanted reactions consisting in urticaria, and wheezing<sup>14</sup>.

SIT treatment of IgE-mediated disorders has proven to be successful when carefully conducted, and is the only treatment of respiratory allergy which can effectively cure the child<sup>14,20,21</sup>. Although its mode of action is less obscure than in the past, the only rational progress that SIT has undergone during its long life has been due to the reduction of patient sensitivity for a given allergen<sup>22</sup>.

As a matter of fact, SIT is a rapidly reputed therapeutic method, especially in the last 15 years, after the allergen purification. Recently, there has been an increasing awareness about characterization and standardization of allergenic extracts used for a beneficial treatment. The considerable evidence that SIT will increase tolerance to aeroallergens in many children, as emerged from recent contributions will surely allow to feed our current SIT knowledge to further amplify its effectiveness on natural history of respiratory allergies, hence promoting resolution of several demands, although its mechanism of action has not been fully elucidated. SIT employment in treating respiratory allergies continue to be a critical issue, despite SIT represents the only specific treatment of such allergies<sup>23</sup>.

Several progresses in the immunological field have allowed to demonstrate several SIT-induced immunological changes, both humoral and cell-mediated. SIT favorable effects may be caused by a variety of humoral immunological changes, such as rise of blocking antibodies belonging to IgG class, with notable affinity for specific allergens<sup>24</sup>, which they can bind to in competition with IgE antibodies<sup>25</sup>; increasing suppression of seasonal IgE increase<sup>23</sup> and slow decline of

its level over years<sup>26</sup>, suppression of allergen-specific secondary IgE response<sup>23</sup>, induction of specific T-suppressor cells<sup>27</sup> and development of anti-idiotypic antibodies<sup>28</sup>. The main cell-mediated immunological changes include reduced skin late phase response to specific allergens<sup>29</sup> and reduced T lymphocyte proliferation and cytokine synthesis in response to allergens<sup>16</sup>; reduced sensitivity of basophils<sup>23</sup> as well as of production of chemotactic factors for eosinophils and neutrophils<sup>31</sup>, potential switching from Th2 to Th1 T cells<sup>16</sup>, and reduced expression of low affinity receptors for IgE (CD23) on peripheral B cells<sup>17</sup>. The net result is suppression or prevention of IL<sub>4</sub> synthesis by T cells in allergic individuals, which is of pivotal significance for successful SIT.

SIT induces IgG production<sup>24,25</sup>. Such blocking antibodies inhibit specific allergen binding to target cells in the shock organs, and activation of either high affinity receptor for IgE, or consequently mast cell, or mediator release. An increase of specific IgG after SIT is due to a relatively large proportion of the IgG<sub>4</sub> subclass, even after 9 months<sup>3</sup>. Another possible mechanism through which SIT may influence the allergen responses is the stimulation of IgG and IgA blocking antibodies in respiratory secretions. This theory appears to be confirmed in patients with AR<sup>25</sup>.

As previously alluded to, several controlled pediatric studies<sup>1-14</sup> have demonstrated the remarkable effectiveness of SIT in positively influencing the natural history of pediatric asthma.

## Materials and Methods

The study population included 56 children (15 males and 13 females), aged between 3 and 6 years (mean 4.1 years). A second group included 28 control children with comparable clinical characteristics such as sex and age. The children of both groups were consecutively followed at the Allergy and Clinical Immunology Division, Department of Pediatrics, University of Rome "La Sapienza", all with the following prerequisites:

- 1) to suffer from allergic pollen-induced oculorhinitis and/or asthma since at least 3 years;
- 2) allergic symptoms present for at least 20-30 days during grass-pollen pollination seasons in the previous years;
- 3) symptom remission outside of grass-pollen pollination periods;
- 4) having never been subjected to SIT;
- 5) having not received an appreciable benefit, in the previous seasons, from preventative treatments using DSCG or Nedocromil sodium, or ketotifen, with the consequent necessity of using a symptomatic treatment with antihistamines, bronchodilators and/or corticosteroids;
- 6) residence in Rome county;
- 7) SPTs and RAST negative for molds, dermatophagoides;
- 8) not suffering from any other important disease

We assessed whether the babies were "at risk" of atopic disease because of a positive family history of atopy since one or both parents and/or other siblings suffered from asthma, or AD, or AR. The diagnosis of atopic diseases in the children was done according the following criteria: clinical history, physical examination and positive skin prick tests (SPTs) and/or RAST to the most common inhalant allergens.

#### **Skin Prick Test**

Appropriate emergency equipment and medications were available on site. Antihistamine drugs and topical steroids were stopped at least 2 weeks before the application of the SPTs. Skin testing was done at baseline by the prick method on the volar surface of the forearm by a trained in allergy doctor with the co-operation of a qualified nurse. The skin was marked with a ballpoint pen for the allergens to be tested. The babies were then tested with: histamine hydrochloride (1 mg/ml) as a positive control and isotonic saline as a negative control. We continued with a battery of food and inhalant allergens, including whole CM protein, casein, lactalbumin, egg, wheat, fish, soy, *Dermatophagoides pteronyssinus*, *Alternaria alternata*, *Lolium perenne*, *Cynodon dactylon*, *Olea europea* and *Parietaria offici-*

*nalis* (SARM, Roma, Italy). The diagnostic extract of each individual allergen was placed on the volar surface of the forearm as drops through which the skin was superficially pricked with a straight pin for one second. A new pin was used for each prick test and then discarded, and the drop of the extract was then wiped off about one minute after the prick<sup>33</sup>.

SPTs were read at 20 minutes and considered positive as follows:

- + when the wheal was the half of the histamine wheal;
- ++ when the wheal was equal to the histamine wheal;
- +++ when the wheal was two-fold the histamine wheal;
- ++++ when the wheal was more than two-fold the histamine wheal<sup>34</sup>.

We took for positive only children with a +++ or ++++ reaction, that is a wheal  $\geq 3$  mm with an area = 7 mm<sup>2</sup> (cut-off). So we considered as positive only the children with a mean wheal diameter of 3 mm or larger than the negative (saline) control. A positive (histamine) control was performed to ensure the absence of any antihistamine drug interference<sup>35</sup>.

#### **Total IgE**

Determination of total serum IgE levels was done by paper radioimmunosorbent test (PRIST, Pharmacia Diagnostics AB, Sweden), and results were expressed in kU/L.

Specific IgE antibodies and determination of specific IgE levels was done by radioallergosorbent test (Phadezym RAST, Pharmacia Diagnostics AB, Sweden).

RAST results are expressed in RAST Units (PRU = Phadebas Rast Unit) in International Units (IU) per ml as follows:

- 1<sup>st</sup> class = IgE levels < 0,35 IU/ml,
- 2<sup>nd</sup> class = IgE levels > 0,35 IU/ml and less than 0,7 IU/ml,
- 3<sup>rd</sup> class = IgE levels between 0,7 IU/ml and 17 IU/ml,
- 4<sup>th</sup> class = IgE levels higher than 17 IU/ml.

Only RAST results > 0,35 IU/ml were considered positive.

IgG antibodies were measured with nephelometry and expressed in kU/L.

For the diagnosis of asthma, 3 episodes of wheezing without fever were required.

For the diagnosis of rhinitis, nasal discharge and/or blockage occurring continuously for at least 4 weeks plus the typical pale aspect of allergic mucosa on rhinoscopy, without any sign of infective rhinitis in other relatives was required.

The children were not subjected to bronchoprovocation studies owing to their young age.

### **Study Group**

All children lived in Rome city, where the grass-pollen season starts at the beginning of March and continues until the end of June. Therefore, when the patients were enrolled, the mean daily grass-pollen count was 85 grains/mq<sup>3</sup> of air, and increased up to 110 during the month of May and the first week of June when the study was done (Figure 1).

The 56 children were randomized and assigned to two groups: the first group has received an alum precipitated extract containing a single allergen: Grass (SARM, Roma, Italy), expressed in BU (Biological Units) and standardized, insuring uniformity and reproducibility among different batches of the same allergenic extract. The control children were cared for by their general practitioners or pediatricians, and were followed medically as the study group.

### **Informed Consent**

The parents of all children gave an informed consent.

### **SIT Administration**

SIT was prescribed by the investigators of this study, or occasionally by other physicians, supervised by the same investigators. SIT was prescribed and administered according to the guidelines of the European Academy of Allergology and Clinical Immunology (EAACI)<sup>36</sup>.

### **Diary Cards**

The parents were provided with diary charts where they kept a record of the days and nights with asthma and drug usage.

The doctors had other diary charts where each time they noted the date, the amount of the allergenic extract dose, the type of possible systemic reaction, the time of onset of symp-

toms, the severity of the systemic reaction, its duration and the type of emergency treatment and the outcome. To every symptom was assigned an arbitrary score from 0 = absent, 1 = mild, 2 = severe. It was therefore achievable to correlate the symptom trends to either the airway concentrations of grass pollens, or the feasible drug assumption (Figure 1).

The children were observed for 30 minutes following the treatment<sup>37</sup>. Facilities for emergency treatment were at immediate disposal<sup>36</sup>.

The children were followed four times in the first year, three times in the second and third year and subsequently two or three times depending on the necessity, always bringing the proper diary charts reviewed by the doctor.

During the pollen season (april-may) the patients could be treated, in case of necessity, with metered dose pressurized aerosol delivering antihistamines, bronchodilators and/or corticosteroids.

### **Statistic Study**

The statistical calculations were performed using the X<sub>2</sub> test.

## **Results**

All children tested positive only for pollen allergens, *Lolium perenne*, *Cynodon dactylon*, *Olea europea* and *Parietaria officinalis*.

On entry to the trial both groups were similar in severity of ocularrhinitic and/or asthmatic symptoms recorded on diary cards during the baseline period. The analysis of children symptoms scores recorded at home on diary cards showed a statistically significant difference in favour of SIT treatment ( $p = 0.0001$ ) compared to control children. Drug usage was significantly lower (Fisher = 0.0219) in SIT-treated children (5/14 of whom 3 discontinuously and 2 continuously), compared to control children (11/14 of whom 3 discontinuously and 8 continuously) (Figure 2).

Specific IgE values determined at the start and the end of this study in SIT-treated children were significantly decreased from 26,48 KU/L (before treatment) to 23.06 KU/L (after treatment), whereas the values were almost similar in the control group: IgE = 25,70 KU/L (before treatment), IgE = 24.55 KU/L

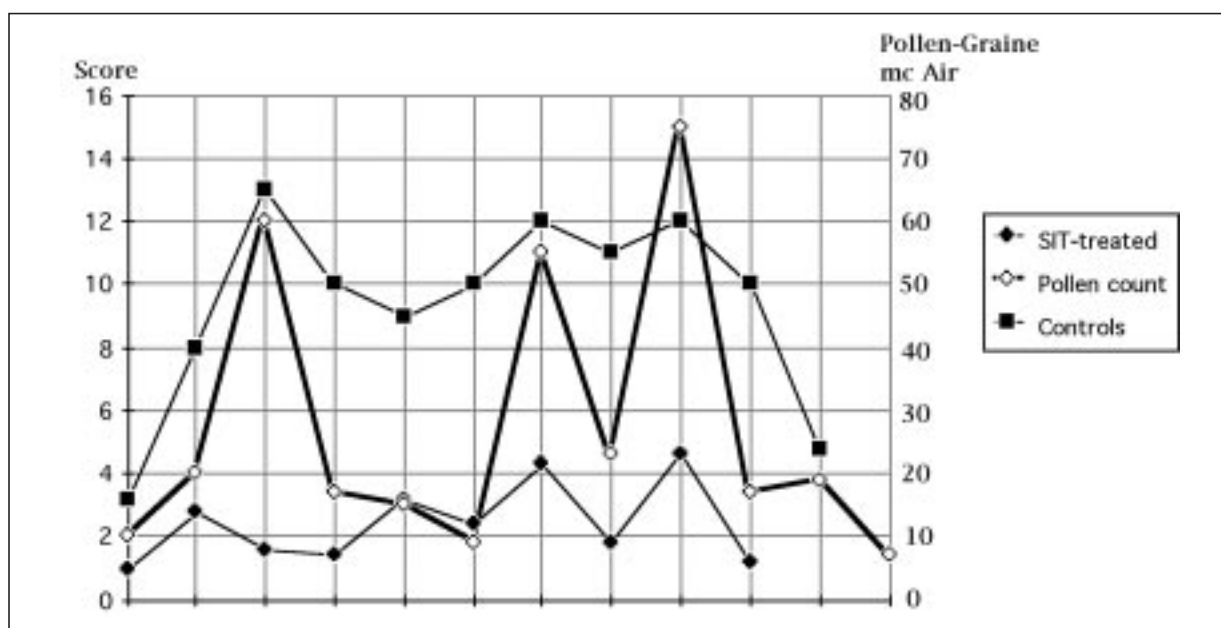


**Figure 1.** Symptom rate (week mean) during Grass pollen season in 56 children aged between 3 and 6 years with respiratory allergy treated with SIT and controls. Follow-up: 3 years

(after treatment) ( $p = 0.0001$ ). In particular, IgG antibodies significantly increased in the study group, whereas they were almost unchanged in the control children ( $p = 0.0001$ ) (Figure 3).

SIT has been well tolerated and only 2 children presented unwanted reactions consisting in urticaria<sup>1</sup>, and wheezing<sup>1</sup>. These effects were caused by hyperdosage and cleared in all children, reducing the dose.

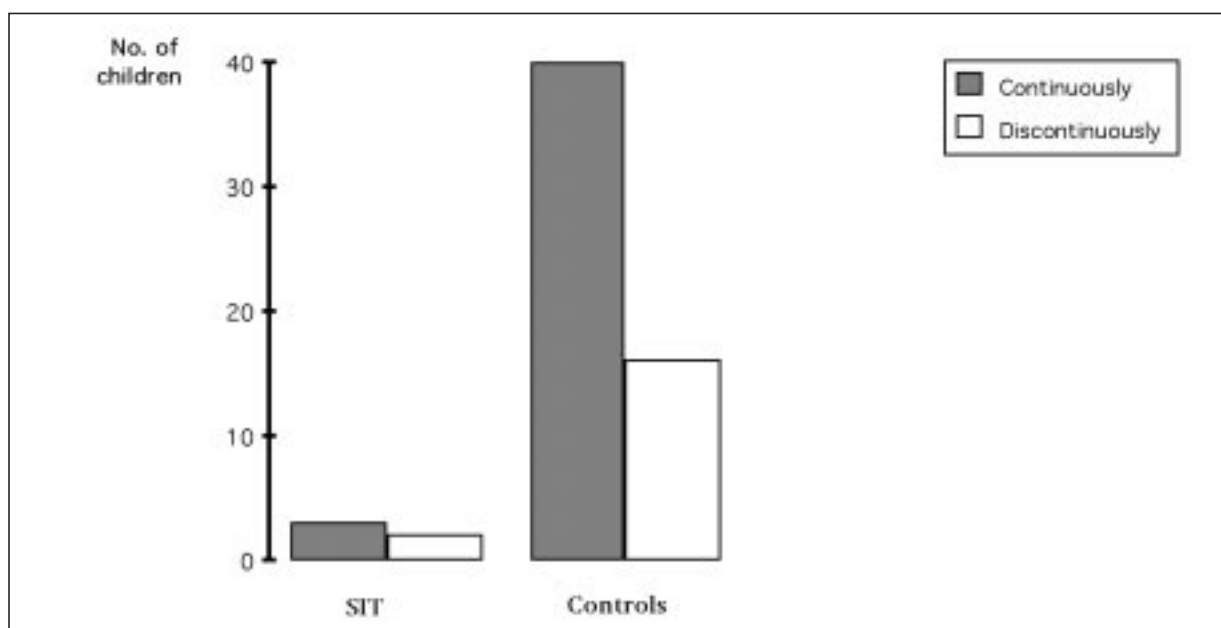


**Figure 2.** Number of children treated continuously or discontinuously with anti-histamine in the study group and controls.

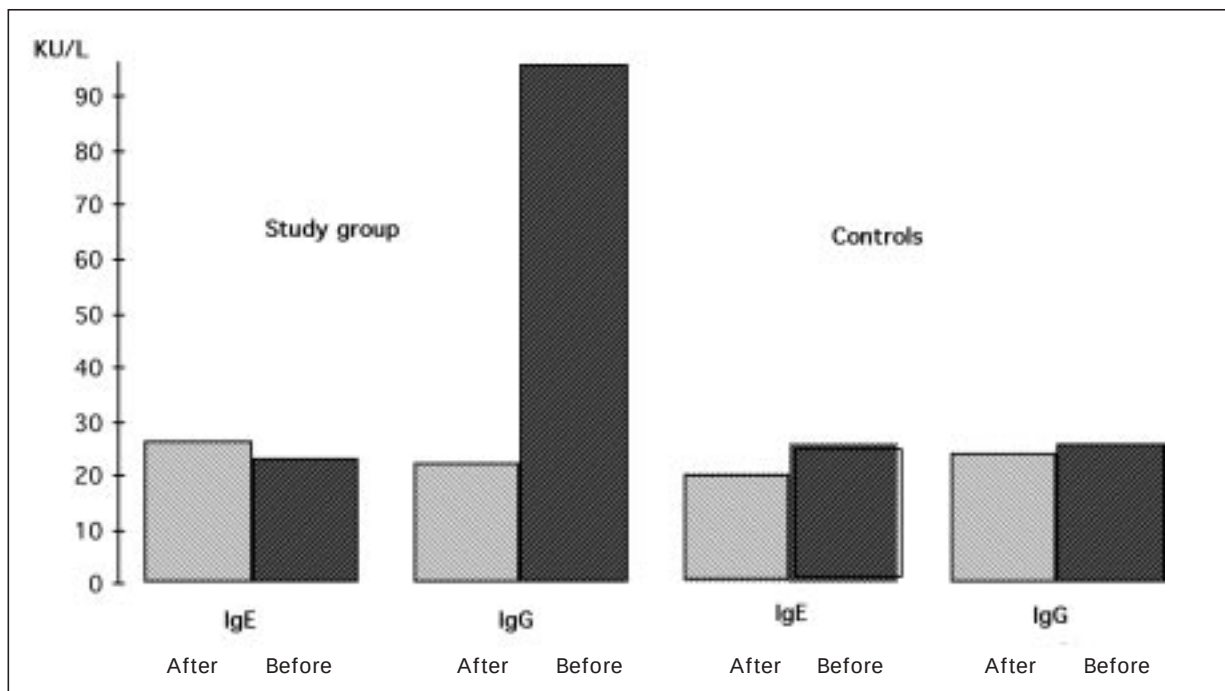


**Figure 3.** IgE and IgG antibody levels before and after SIT in 28 children and 28 controls aged between 3 and 6 years with respiratory allergy. Follow-up: 3 years. IgE and IgG levels before vs after SIT:  $p = 0.00001$ .

### Discussion

SIT should be initiated very early, or within a short period from the onset of respiratory symptoms, in order to prevent the chronic allergic condition from occurring<sup>32</sup>. Why it is so necessary to begin SIT early in life? To stop the atopic march. A meta-analysis of ours revealed that asthma onset within the first year is certain in 34.5-56.2% of babies, and a greater proportion (82.4%) is manifest between the 4th and the 7th year of life. Further, asthma appearance is evident in 90% of children aged 8 years or less<sup>38</sup>; therefore the most severe cases have an early onset, and the allergic component of asthma is most pronounced in children and adolescents<sup>39</sup>. In addition, even slight, newly diagnosed asthma is accompanied by features of ongoing mucosal inflammation and desquamation of bronchial epithelium<sup>40</sup>, and elastic fibers in the bronchial walls are destroyed by long-standing asthma<sup>39</sup>. Bronchoalveolar lavage findings and mucosal biopsies in asthmatic children aged 1-15 years have revealed bronchial inflammation and collagen deposition below the basement

membrane, showing that both inflammation and remodelling occur early in life<sup>41,42</sup>. Before the damage takes place the main option, SIT, should not be disregarded<sup>43,44</sup>. Because during SIT injections side effects of various extent (local, diffused or systemic) can take place, we have demonstrated that anaphylactic reactions are extremely rare in children: a fatal reaction with no known error occurred only in three children<sup>20</sup>, while in 1,313 children with asthma or AR treated by us there were no significant reactions<sup>4,5,14,20</sup>; only severe reactions in 0.09% of injections and one case of shock = 0.0016% of injections and in 0.089% of 1,119 treatments<sup>19</sup>. However, there may be a known error of SIT administration, or an incorrect dose of epinephrine, an error of dosage and delay in the treatment, or children fail to wait in the doctor's office<sup>45</sup>. Rarely, injections may be done in children with wheezing<sup>45</sup>, but in the great majority of cases there is no known error in the SIT administration. It is therefore necessary that SIT should be always prescribed by specialists and administered by physicians trained to manage systemic reactions if anaphylaxis occurs.

Traditional subcutaneous SIT has been shown to be the elective form of treatment of respiratory allergies. It is true that three studies with sublingual oral SIT claim efficacy. In a preliminary study on 8-12 years-old asthmatic children sensible to Der p has demonstrated a statistically significant reduction of clinical manifestations and an equally significant IgG4 increase<sup>46</sup>. Additional positive results were shown in children allergic to Der p<sup>47</sup>, pollens<sup>48</sup> and *Parietaria*<sup>46</sup>. We are waiting for additional studies on sublingual oral SIT to evaluate its effectiveness.

It has been hypothesized that children are more sensitive and reactive to SIT than do adults<sup>39</sup>, however the risk of adverse reactions is actually by far less in infants and children than in adults: their parents supervise and record any apparently harmful allergic symptoms of the child<sup>39</sup>. Furthermore, if parents are opportunely instructed, they will report to doctors on any allergic reaction to SIT, including intercurrent infections, or possible massive allergen exposures, either representing temporary contraindications to SIT<sup>21</sup>; Children tolerate potential antishock therapies better than adults, in particular epinephrine as in the case of anaphylactic shock, whereas adults with coronary heart disease have higher chance of suffering from a coronary harm if given epinephrine<sup>48</sup>. In addition they have along the walls of coronary arteries, a greater number of mast cells which upon challenge may release mediators into the myocardium. Therefore, immediate epinephrine administration to infants and children is the most important life-saving measure<sup>49</sup>.

These data suggest that if suitable allergen extracts are used, the therapeutic indications are exactly followed, and children are followed by their doctors as frequently as required, SIT is effective in the treatment of pediatric asthma and the reactions are scarce. A recent Position Paper<sup>50</sup>, an International Conference on Allergic Rhinitis in Childhood<sup>51</sup>, and a Symposium on Specific Allergy<sup>52</sup> all seem to have disregarded that SIT has been performed in 29 studies encompassing 2,024 children with asthma and/or AR, and as many controls, 27 of which highly positive ( $p < 0.0001$ )<sup>14</sup>. However, several recent papers make it impossible to overlook SIT<sup>53-55</sup>, especially because it is confirmed that the possible systemic reactions are surely

nonfatal<sup>56</sup>, and that the immune decrease of type 2 CD4+ and CD8+ T cells might be closely correlated with the SIT regulatory mechanisms<sup>57</sup>. The quality of life may be an important reference for assessing the impact of disease morbidity on daily life activities<sup>58</sup>. A risk factor for development of asthma in children and adolescents has been demonstrated, that is the wheezing that persisted from childhood to adulthood or that relapsed after remission in more than 25% of children<sup>59</sup>. So the clinician may conclude that at variance with drugs (Figure 2), SIT is a preventive treatment which has been shown to have long-term efficacy even several years after withdrawal.

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