Immunotherapy of Children with Rhinoconjunctivitis due to Birch Pollinosis

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ABSTRACT

Immunotherapy of Children with Rhinoconjunctivitis due to Birch Pollinosis

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In this investigation of immunotherapy (IT) children 6–16 years old with rhinoconjunctivitis due to birch pollinosis were included.

I. Methodological studies. To monitor IT a reliable provocation test is desirable. The conjunctival provocation test (CPT) was evaluated in 20 children with four repeated challenges. The test was found to have a good precision, it was simple and appeared to be clinically safe. After repeated tests the levels of IgE antibodies against birch increased considerably in three children, indicating an immunological response.

A pollen peak affects the symptoms of an atopic individual for several days. Thus pollen counts for previous days must be taken into account when relating symptom scores with the counts. A dynamic time series model was therefore developed by which groups of atopic patients could be compared when exposed to different amounts of pollens.

II. Cross-reactivity between deciduous trees during IT. Immunotherapy with pollen allergen preparations made from either birch (B) or a mixture of birch, alder and hazel (M) were compared. As measured with symptom scores the children in the M group improved at least as much as those in the B group. In the B group but not in the M group the improvement correlated with immunochemical findings before IT or early during the treatment, probably an unsignificant finding. Otherwise there was little difference between the two groups. Analysis of sera with crossed radioimmunoalectrophoresis in 20 children revealed that 60% of the children below 13 years had developed IgE antibodies during IT against allergens against which they had not been allergic before IT. This had no appearent clinical implications.

III. Oral immunotherapy (OIT). A pilot study of 18 children treated with high doses of a birch pollen allergen preparation in enteric coated capsules and 8 untreated controls indicated that OIT was effective as shown by lower symptom scores, less conjunctival sensitivity and increased levels of IgE antibodies against birch. However, the gastrointestinal side-effects were pronounced. Therefore a second double-blind study, in 30 children, was performed reducing the side-effects through a different dose schedule. Compared with the placebo group, the actively treated children had lower symptom scores (p = 0.04), reduced skin sensitivity (p = 0.01), increasing levels of IgE (p = 0.001) and IgG (p = 0.007) antibodies against birch before the birch pollen season and a suppression of the seasonal increase in levels of IgE antibodies against birch (p <0.001). After three months of OIT but not after ten months they also had a lower sensitivity in CPT than the controls (p = 0.01).

The intestinal permeability as assessed by the urinary recovery of differently-sized polyethylene glycols was studied in 24 of the children during IT. No changes were seen in the group of actively treated children. In two additional children openly treated with OIT small bowel biopsies were taken with normal morphological findings. Thus OIT did not result in a generalized inflammation of the small bowel.

Key words: Birch pollinosis, Conjunctival provocation test, Crossed radioimmunoelectrophoresis, Crossreactivity tree pollens, Immunotherapy, Oral immunotherapy, Polyethylene glycol absorption, Pollen counts, Rhinoconjunctivitis, Symptom score
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To all the Wonderful Children who have participated in these studies
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The intestinal permeability as assessed by the urinary recovery of differently-sized polyethyleneglycols was studied in 24 of the children during IT. No changes were seen in the group of actively treated children. In two additional children openly treated with OIT small bowel biopsies were taken with normal morphological findings. Thus OIT did not result in a generalized inflammation of the small bowel.

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ABBREVIATIONS

B The pollen allergen preparation made from birch only or the group of children treated with this preparation
BU Biological units
CIE Crossed immunoelectrophoresis
CPT Conjunctival provocation test
CRIE Crossed radioimmunoelectrophoresis
HEP Histamin equivalent in skin prick test
IgE Immunoglobulin E
IgG Immunoglobulin G
IT Immunotherapy
LIT Local immunotherapy
M The pollen allergen preparation made from a mixture of birch, alder and hazel or the group of children treated with this preparation
OIT Oral immunotherapy
PEG Polyethyleneglycols
RAST Radioallergosorbent test
SIT Subcutaneous immunotherapy
SPT Skin prick test
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This thesis is based on the following papers which will be referred to in the text by their Roman numerals:


II. Göran Broström & Christian Möller: A new method to relate symptom scores with pollen counts. A dynamic model for comparison of treatments of pollen allergy. (In manuscript).

III. Christian Möller & Sten Dreborg: Crossreactivity between deciduous trees during immunotherapy. I. *In vivo* results. (Accepted for publication in Clin Allergy).

IV. Christian Möller, Sten Dreborg & Roland Einarsson: Cross reactivity between deciduous trees during immunotherapy. II. *In vitro* results (In manuscript).

V. Bengt Björkstén, Christian Möller, Ulf Broberger, Staffan Ahlstedt, Sten Dreborg, S. Gunnar O. Johansson, Per Juto & Åke Lanner: Clinical and immunological response to oral immunotherapy with a standardized birch pollen extract. (Accepted for publication in Allergy).

VI. Christian Möller, Sten Dreborg, Åke Lanner & Bengt Björkstén: Oral immunotherapy of children with rhinoconjunctivitis due to birch pollen allergy. A double blind study. (Accepted for publication in Allergy).

VII. Christian Möller, Karl-Eric Magnusson, Tommy Sundqvist & Bengt Björkstén: Intestinal permeability as assessed with polyethyleneglycos (PEG 400 and PEG 1000) in birch pollen allergic children undergoing oral immunotherapy (Accepted for publication in Allergy).
INTRODUCTION

Immunotherapy is one of the cornerstones in therapeutic arsenal of the allergist. Although performed since the beginning of this century the first properly controlled study of IT was not carried out until 1962 (Lowell & Frankland 1965). Thereafter several trials have demonstrated the efficacy of IT (Sørensen 1976; Norman 1978; Rocklin 1983). The underlying cause for the improvement during IT remains, however, unexplained. Recently immunochemical methods such as RAST and CRIE, and better defined and more potent allergen preparations have made it possible to better investigate the mechanisms of IT and to meliorate the treatment. The present studies deal with these possibilities.

Definitions

In this paper the term hypersensitivity is defined as a condition of unusual sensitivity to a substance or substances which in like amounts do not affect others. Allergy is hypersensitivity based on immunological mechanisms. An element which induces an immune response is called an antigen. An allergen is an antigen which induces an allergic response. If the allergic person has specific antibodies of type IgE against allergens which may provoke the allergic symptoms the condition is called atopy and the disease atopic.

Substances such as pollens, animal danders, mites, moulds etc. are often called allergens as they are capable of inducing allergic symptoms in sensitive patients. As these substances often consist of several different molecules the term “allergen” is used more restrictively in this work, i.e. it is confined to those molecules which bind IgE antibodies. An allergen preparation for diagnostic or therapeutic use may contain one or several allergens. In such a preparation a major allergen is an allergen to which a majority of the relevant patient sera bind in CRIE and at least 25% of the sera cause strong radiostaining. In contrast a minor allergen is an allergen to which only 10% or less of the relevant patient sera bind. An intermediate allergen is thus an allergen of importance between the major and the minor allergens (Aukrust & Borch 1980).

Incidence of atopic diseases

Atopic diseases appear to have been uncommon before this century (Phoebus 1862) and probably the incidence has been increasing in all industrialized countries, at least up to the last decade. In reports from 1960–1971 the incidence of hayfever among school-children was <1% in Glasgow, 2.7% in Finland, 3.1% in Iowa and 19% in Colorado (Smith 1974). The incidence of asthma varied from 2.1% in Finland through Iowa, Colorado, Houston, Scotland, Switzerland, Maryland, New Zealand, England, Australia and up to 19% in Mitchigan (Smith 1974). The figures are, however, not comparable neither for hayfever nor for asthma as the methods for collection of data differ and sometimes cumulative incidence is given rather than incidence. Furthermore “wheezing”, which is common particularly in infants, is sometimes included in the figures for asthma and sometimes not. But despite these reservations there are at least two reports indicating a true increase (Smith 1976, Eaton 1979).

Airway allergy has been thought to be less common in developing countries than in industrialized societies. The incidence of asthma has thus been reported to be low in e.g. India, among Mexicans working in Houston, in Kenya and in northern Nigeria (Smith 1974, Mitchell 1970, Warell et al 1974, Abdurraham & Taqi 1982). The discrepancy between the industrialized and
the developing countries has been clearly demonstrated in two studies in which the same ethnic group was investigated under different conditions (Smith 1976; van Niekerk et al 1979), suggesting that some exogenic factor acting during early life is important. In contrast there are several studies indicating a high incidence of asthma in other developing countries such as Tanzania, southern Nigeria and Venezuela (Carswell et al 1977, Aderle 1979, Lynch et al 1984). The incidence of atopic diseases is probably increasing in developing countries (Anderson 1974, Woolcock et al 1981, Dowse et al 1985, Ellul-Micallef & Al-Ali 1984).

Atopic diseases among pets is an increasing problem in industrialized countries (Öhlén 1985) possibly as a result of the animals being subject to similar unknown environmental factors as their owners.

In Sweden also the incidence of atopy appears to be increasing. Among school-children it was reported that 2.4% had hayfever in 1960 (Engström et al 1960), 1.4% had asthma in 1953 (Kraepelin 1953) and in 1973 3.8% had rhinoconjunctivitis and 2.7% had asthma (Kjellman 1976). The cumulative incidence for rhinoconjunctivitis was 8.9% in 1980 and for asthma 5.1% (Åberg 1982). In another investigation of children 7–9 years old from the same time the cumulative incidence for rhinoconjunctivitis was 5.1% and for asthma 2.1% (Kjellman et al 1982). Among conscripts an increase of allergic diseases has been noted from 1971 to 1981 employing similar methodology (Åberg 1984).

Fig. 1. The geographic site of Umeå.

<table>
<thead>
<tr>
<th>Year of birth (years of age)</th>
<th>Asthma</th>
<th>Rhinoconjunctivitis</th>
<th>Excema</th>
<th>Urticaria</th>
<th>Other allergy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1969 (6–7)</td>
<td>6.6</td>
<td>13.6</td>
<td>14.8</td>
<td>13.0</td>
<td>&lt;1</td>
</tr>
<tr>
<td>1968 (7–8)</td>
<td>8.8</td>
<td>16.4</td>
<td>14.4</td>
<td>14.7</td>
<td>11.7</td>
</tr>
<tr>
<td>1967 (8–9)</td>
<td>4.3</td>
<td>10.7</td>
<td>15.1</td>
<td>12.3</td>
<td>14.7</td>
</tr>
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It has also been noted both among school-children and conscripts that atopy during the last decade in Sweden is more frequent in the northern part of the kingdom where Umeå is situated than in southern Sweden (Fig. 1; Åberg 1982 and 1984). This is in line with a study from Umeå in 1976 (Möller: Unpublished data). All 3 417 children born in 1967–1969 (the children were thus 6–10 years old) were asked if they had or had had symptoms indicating allergy. Answers were obtained from 99.5% of the parents and showed a cumulated incidence of 34.1% (Table I). Those with asthma were asked about the severity of their symptoms and 2.5% had had asthma but were now free of symptoms while 4% claimed that they had present asthma. A random sample among those with rhinoconjunctivitis indicated that about 10% of the 3 417 children had present symptoms. The frequency of rhinoconjunctivitis was related to age being higher in older children than in younger indicating that symptoms of rhinoconjunctivitis often start during the first school years (Table I). The frequency of allergic symptoms in parents and siblings was similar irrespective of the child’s symptom, i.e. asthma, rhinoconjunctivitis, excema or urticaria (Table II). Table II also demonstrates that it is common with allergic symptoms in the family even if the child is free from a specified allergic symptom. The incidence of airway allergy and particular rhinoconjunctivitis in this study was much higher than in the previously mentioned report by Kjellman from southern Sweden (1976). The reason for this is unknown. However, in Umeå the material which most commonly gives allergic symptoms is pollens from birch and other deciduous trees in contrast to southern Sweden where grass pollen allergy is more common (Eriksson 1977; Hultquist & Kjellman 1981). The birch pollen season in Umeå is more intense than in the south of Sweden (Rosenhall et al. 1984) and even though this difference is moderate it may offer a possible explanation for the high prevalence of airway allergy in Umeå. It has been claimed that additives to foods, air pollution (Smith 1974), poor breast-feeding (Grulee & Sandford 1936; Björkstén 1983) or absence of parasites (Grove 1982) has resulted in more atopic diseases in the industrialized part


<table>
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<th>Symptom in the child</th>
<th>Allergy in any parent</th>
<th>Allergy in any sibling</th>
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<tr>
<td>Asthma</td>
<td>47.7</td>
<td>34.2</td>
</tr>
<tr>
<td>Not asthma</td>
<td>27.8</td>
<td>19.4</td>
</tr>
<tr>
<td>Rhinoconjunctivitis</td>
<td>51.7</td>
<td>27.1</td>
</tr>
<tr>
<td>Not rhinoconjunctivitis</td>
<td>25.7</td>
<td>19.4</td>
</tr>
<tr>
<td>Excema</td>
<td>47.5</td>
<td>30.4</td>
</tr>
<tr>
<td>Not excema</td>
<td>26.6</td>
<td>18.7</td>
</tr>
<tr>
<td>Urticaria</td>
<td>45.1</td>
<td>28.0</td>
</tr>
<tr>
<td>Not urticaria</td>
<td>26.8</td>
<td>19.3</td>
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of the world but for none of these factors is the situation in northern Sweden unfavourable compared to southern Sweden (Persson 1984). Other suggested, but uncontrolled and unlikely, explanations for the high incidence in Umeå would include genetic differences, pets being more common, different eating habits, smoking being more frequent (Zetterström et al 1981), dry indoor climate and the long winter making the children stay indoors more than in the south. The high incidence of hayfever in Finland (Malmberg 1979) further emphasizes the possibility of an allergy promoting environmental factor in the north.

**Immunotherapy of atopic asthma and rhinoconjunctivitis**

The treatment of atopy usually includes elimination of the offending allergen, symptomatic medication and IT. Total allergen elimination is in most cases impossible for an allergen material like birch pollen and is not considered to cure the immunoregulatory defect as a rule. Medication has several drawbacks as it may be ineffective, give severe side-effects, be difficult to take and is expensive if the treatment is intended for many years. Nor would symptomatic medication be expected to cure the disease. It is well-known that properly performed immunotherapy gives relief to at least some degree when given to patients allergic to pollens (Sörensen 1976, Norman 1978, Rocklin 1983), mites (Mosbech 1985), animal danders (Valovirta et al 1983; Löwenstein et al 1984), Cladosporium (Koivikko et al 1983) and Hymenoptera venoms (Hunt et al 1976). The treatment is, however, time-consuming, expensive, may sometimes be dangerous and does not always yield satisfactory results. In addition it should be noted that it is not known whether even properly performed IT gives a permanent improvement in the majority of patients after the cessation of therapy. Such information can only be obtained after prolonged experience with the new generation of standardized and potent allergen preparations for IT.

Several criteria should be fulfilled in the selection of patients for IT. The allergy should be essential for the symptoms, the allergen preparation used for the treatment should contain all relevant allergens in sufficiently high concentrations, the doses given should be high enough to give the desired immune response and the treatment should continue for a considerable time, *i.e.* for years (Lichtenstein et al 1968, Johnstone & Dutton 1968, Sörensen 1976, Norman 1978, Rocklin 1983). Conventional IT with repeated subcutaneous injections is costly during the treatment period as the allergen preparations are expensive and the therapy requires substantial involvement from the responsible doctors, nurses and patients. Anaphylaxis is a threat after every injection. The long term side-effects of IT are not known but — theoretically — different immunological disturbances may be induced (Rocklin 1983). It has also been advocated that injections of allergens against which the patient is not allergic may give rise to production of IgE antibodies against these allergens (Richter 1958 et al, Greenert 1971 et al, Marsh 1972 et al, Hunt et al 1978, Turkeltaub et al 1978). If this is common and if these "new sensitivities" are clinically important (Turkeltaub et al 1978) it is essential to give IT with only the allergens against which the patient has developed IgE antibodies.

These considerations have helped to fortify the search for more purified allergen preparations containing only the relevant allergens and with all non-allergenic material removed. It is well-known since many years that allergen preparations from various grasses cross-react (Rackemann et al 1936; Bernstein et al 1976; Löwenstein 1980; Dirksen & Österballe 1980). This is also true for extracts from deciduous trees (Rackemann et al 1936; Juhlin-Dannfelt 1944; Zetterström et al 1972; Bernstein et al 1976; Löwenstein 1980; Dirksen & Österballe 1980; van Dalen & Voorhorst 1981; Eriksson 1978b; Iepsen, Böwadt et al 1985). If this cross-reactivity were complete it would be sufficient to use an allergen preparation from only one species within each group of plants for diagnosis and treatment of allergy against all the members of the
group. Even if the cross-reactivity is only partial, treatment with an allergen preparation made from only one species may yield good results as it is possible that only certain major allergens shared by several species are necessary for IT. Under such circumstances the use of an allergen preparation made from only one species would diminish the risk of developing "new sensitivities" as fewer potential allergens could be given. A far-reaching attempt to do this was recently done using a highly purified timothy preparation containing only the two most important allergens (Österballe et al 1982). This preparation was, however, less effective than a partly purified timothy pollen preparation in SIT of patients with grass pollinosis. On the other hand a partly purified timothy pollen preparation seems to be superior to a crude grass pollen preparation (Berg et al 1980, Frostad et al 1983, Kjellman 1983a). Recently it was shown that a partly purified grass pollen preparation made from five different grasses was superior to a partly purified timothy preparation (Kjellman 1983b). Allergen preparations tailored for the individual patient, containing only the allergens against which the patient has developed IgE antibodies, could be an interesting possibility in the future but this is probably not economically possible with present technology.

The cross-reactivity for deciduous trees is less complete than for grasses (Bernstein et al 1976, Löwenstein 1980, Dirksen & Österballe 1980). Most patients with this type of allergy, living in an area where birches are common, react against birch and it is uncommon to find patients allergic exclusively to other deciduous trees in such areas (Rackemann et al 1936; Juhlin-Dannfelt 1944; Eriksson et al 1984). Thus it appears as if most of the relevant allergens for deciduous trees are found in birch pollen.

The risk for anaphylaxis during IT has resulted in the search for allergen preparations with reduced allergenicity but high immunogenicity. Trials have been done or are presently performed of allergens treated with low molecular weight substances such as formaldehyde and glutaraldehyde, and high molecular weight substances such as alginate, poly-D-glutamic acid-D-lycine and polyethylene glycols (Rocklin 1983; Dreborg 1983; Björkstén 1985). The early studies indicate that modified preparations may be associated with reduced side-effects but maybe as effective clinically as unmodified preparations. The precise clinical role of these compounds has yet to be determined.

Local IT of the shock organ is another attempt to reduce the risks of systemic reactions and at the same time avoiding the inconveniences of repeated injections. With low doses of an aqueous allergen preparation or allergens treated with formaldehyde or glutaraldehyde applied into the nostrils in a nebulized form some effect of treatment in atopic rhinitis may be noted (Platts-Mills 1979; Johansson et al 1979, Nickelsen et al 1981, Georgitis et al 1983). A better effect of this LIT could have been expected with higher doses, but then clinical symptoms were triggered abolishing the favorable influence (Platt-Mills 1979). Thus it is often difficult to stimulate the immune system enough to initiate a favourable clinical and immunological response that dampens the allergic reaction without simultaneously risking generalized allergic reactions.

The idea of eating the offending allergens is old (Curtis 1900) and several studies particularly in animal models have shown that the immune system can be stimulated by antigen presented to the gut (Hansson & Brandtzaeg 1980; Bienenstock 1985). The most important consequence of local application of an allergen preparation is possibly a stimulation of mucous membranes not only locally but also at distal sites (Hansson & Branzaeg 1980; Bienenstock 1985). That induction of mucosal antibodies in exocrine glands against antigens presented to the gut without a detectable immune response in the blood, is well-known for microbial antigens (Hansson & Brandtzaeg 1980; Bienenstock 1985). Especially in case of food allergy it has been popular to
treat with increasing amounts of the offending foodstuff orally, but there are no controlled studies showing efficacy (Zanussi 1982). Many authors have reported good results with OIT against inhalant allergens (Curtis 1900; Touart 1922; Thiberge 1942; Stemman et al 1979; Wahn et al 1976; Wortmann 1977). Some have found that the allergen might be destroyed by gastric juice and therefore have used enteric coated capsules designed to dissolve after leaving the stomach (Touart 1922; Thiberge 1942). However, controlled studies indicate that the claimed treatment efficacy is due to a placebo effect (Rebien et al 1982; Urbanek & Gehl 1982; Cooper et al 1984; Taudorf et al 1985). Another way of avoiding the problems with SIT and with the destructing gastric juice is to apply the allergens sublingually in the hope that they will get into contact with the immune system without giving rise to unwanted reactions. This has been tried with discouraging results (Reisman 1981). Ocular or bronchial application of the allergens appear not to have been tried. Other routes of presenting the allergens, e.g. per rectum, are unpractical.

Evaluation of immunotherapy

Elevated production of IgE antibodies is a hallmark of atopic disease. Other immunological findings, e.g. T-lymphocyte abnormalities (Strannegård & Strannegård 1979; Björkstén 1984), are common. The patients are likely to get symptoms when exposed to the allergen. Since the underlying defect is not known IT is an empirical form of treatment even though it has been used since the beginning of the century (Curtis 1900; Sörenssen 1976; Norman 1978). It has been shown that the treatment is specific for the allergen preparation given and that higher doses give a better efficacy (Norman 1978; Rocklin 1983). But high dose IT is associated with a greater risk for generalized reactions (Norman 1980). In properly treated patients symptoms decrease during the treatment period and this effect is possibly lasting. But since the atopic disposition is not affected the symptoms may return, perhaps as a consequence of a new "break-through" (Katz 1978). It has therefore been advocated that IT should continue for ever (Johnstone & Dutton 1968). In scientific work it is, however, difficult to motivate patients for a double-blind placebo-controlled study for a prolonged period with many injections and ethical doubts may be raised against such procedures but, if possible, the evaluation of IT efficacy should continue for several years after the cessation of therapy. It is difficult to do proper double-blinded studies as side-effects of IT are very common when high doses of the allergen are used and therefore actively-treated patients are usually easily distinguished from placebo-treated controls. The difficulty with this and the considerable placebo effect (Sörensen 1976) may be overcome by strictly objective assessment methods — lacking in vivo — with the results evaluated by a blinded investigator without prior contact with the patients. Another demand is that the included patients are well-defined. Despite the difficulties the availability of new allergen preparations have made proper studies of IT more interesting, since higher doses can be given, the content of allergens is controlled, less non-allergenic material is given and modifications may diminish the risk for generalized reactions. To monitor IT several in vivo methods have been used e.g. self-evaluation or examination by the doctor on different occasions, more frequent scoring of symptoms, frequent registration of lung function by the patient at home, registration of the use of symptomatic medication, measurement of the skin sensitivity and the sensitivity of the shock organ as assessed by provocation tests. The allergic symptoms depend on the degree of exposure to the allergens and ideally the allergen exposure should be controlled for every patient and the symptoms could then be related to this exposure.

Some of the in vivo methods such as skin tests and challenge tests have the advantage of giving a directly measurable value but their relevance for the well-being of the patient is less obvious. If the patient has severe symptoms symptomatic medication is often provided. The use of
“escape medication”, if allowed, is an important parameter in evaluation but blunts symptom scoring since it may be difficult to estimate how much relief the medication gives.

In order to monitor IT several *in vitro* tests have been used, *e.g.* basophilic reactivity (Kimura *et al* 1985), histamine release (Sadan *et al* 1969, Norman 1978, Rocklin 1983), lymphocyte function (Rocklin 1983; Möller 1984), lymphocyte receptors (Rocklin 1983), levels of IgE and IgG antibodies against the allergen preparation (Foucard & Johansson 1976; Norman 1978; Rocklin 1983) and allergen specific antibodies of the IgE and IgG isotypes (Djurup & Österballe 1984; Löwenstein *et al* 1984; Nordvall *et al* 1984). Determination of IgE antibodies are of special interest as they, by definition, are associated with atopy. The level of IgE antibodies against a specific allergenic substance increases after allergen exposure and thus pollen allergic patients have varying levels during the year (Berg & Johansson 1971; Deuschl 1976). In a group of patients undergoing IT there is an early rise in IgE against the given allergen preparation followed by a slow decline over years (Foucard & Johansson 1976; Norman 1978; Rocklin 1983; Österballe 1982). The levels of IgG antibodies against the preparation also rise but not as fast as IgE and the increase remains throughout the IT (Foucard & Johansson 1976; Norman 1978; Österballe 1982; Rocklin 1983). In secretions such as tears, nasal secretion, saliva, urine and faeces specific IgA is also of theoretical interest although measurements so far have failed to show any correlation to IT (Deuschl *et al* 1977).

For most of the *in vitro* tests mentioned, the degree of changes during IT correlates roughly to therapy efficacy as measured by symptom scores but for individual patients the correlations are less obvious. According to most authors there are no tests that can be used before IT or during the first months of IT to predict the outcome of therapy. It has been reported that the increase of total IgE, allergen specific IgE or IgG4 or changes in the IgG subclass ratio during the first phase of IT would be predictive (Kjellman *et al*; Österballe *et al* 1983; Søndergaard *et al* 1984) but these results have not yet been confirmed by others.
AIMS

The two primary aims of this work were:

• to study the cross-reactivity between deciduous trees during IT, concentrating on clinical efficacy, possible sensitization against new allergens and the possibilities to predict the outcome of IT (III and IV)

• to study the clinical efficacy and side-effects of OIT (V, VI and VII)

When these aims were approached two questions arose resulting in two secondary aims, \textit{i.e.}:

• to evaluate the precision of CPT as a mean of analyzing the efficacy of IT (I)

• to analyze the relation between symptom scores and pollen counts in patients before and during IT (II)
MATERIAL AND METHODS

Patients

The children included in this work were 6–16 years old when they were asked to participate. They all suffered from rhinoconjunctivitis due to birch pollinosis. The diagnosis of birch pollen allergy was established through a positive case history, a positive SPT and a positive CPT. As blood samples were not analyzed immediately, the finding of circulating IgE antibodies against birch was not an inclusion criterion although all the children were later shown to have such antibodies prior to therapy.

Other allergies, e.g. against grass pollens, animal danders, mites and moulds, were allowed provided they were mild and were not expected to interfere with the evaluation of the birch pollen allergy. The children in the CPT study were allowed even a severe allergy against grass pollens. One child in the cross-reactivity study and one in the OIT study developed a moderate allergy against grass pollens after having been included in the studies but before the start of IT. These children were not excluded. More than 90% of the children had a food hypersensitivity associated with birch pollen allergy, i.e. sensitivity to apples, nuts, raw vegetables etc (Erikson 1978a; Dreborg & Foucard 1983). Mild asthma during the birch pollen season was allowed. Except for allergy the children were healthy.

Allergen preparations

All preparations were manufactured by Pharmacia AB, Uppsala, Sweden. The oak preparation used for tests in the cross-reactivity study and the birch preparation used for therapy in the OIT studies were crude but otherwise all preparations were purified from molecules above approximately 70 000 dalton and below approximately 3 000 dalton. The potencies of the preparations were determined with repeated RAST inhibition tests and compared with reference preparations. The biological activity of these reference preparations was determined with SPT in the HEP system as recommended by the Nordic guidelines (Aas et al 1978) and was expressed in BU. The relative concentrations of the major allergens were alike in all preparations from each tree pollen as verified by CRIE. After these quality controls the preparations were freeze-dried giving them a good stability. The same batches were used throughout each separate study for in vivo tests and treatment except for the cross-reactivity study were the treatment during the last year was done with a new batch.

When the preparations were used for tests or rush IT they were diluted with Albumin diluentR (Pharmacia) containing 0.03% human serum albumine, 0.9% NaCl and 0.4% phenol and when used for maintenance SIT they were diluted with Depot diluentR (Pharmacia) containing 0.2% aluminiumhydroxide. The preparations diluted with Albumin diluentR were used 1–8 days after reconstitution which was well within the limits of stability as claimed by the manufacturer (Zetterström et al 1982).

Pollen counts

Reliable pollen counts are best obtained above buildings as turbulence may otherwise influence the results (Käpylä 1984). The highest roof in Umeå is on the main building of the University Hospital which is situated in an elevated part of the town. A Burkard volumetric pollen trap was placed on the roof 25 meters above the ground. Daily registrations were done during
the scoring periods from April 30 to June 10 each year. During this period mainly birch pol­
lens were captured. In addition some pollens from alder, willow and aspen were recorded. As pollens from deciduous trees cross-react (Rackemann et al 1936; Juhlin-Dannfelt 1944; Zetter­ström et al 1972; Bernstein et al 1976; Eriksson 1978b; Dirksen & Österballe 1980; van Dalen et al 1981; Ipsen, Böwadt et al 1985) all these pollens were added in the calculations. The main alder season during the last two weeks of April was monitored in 1983 and 1984 but the counts were much lower than during the birch pollen period (IV; VII; Rosenhall et al 1984). Hazel does not grow as far north as Umeå. In Umeå there are 3000 planted oaks which otherwise do not grow in the region. They appear to be infertile since no oak pollens were caught in the trap (Rosenhall et al 1984).

**Symptom scoring**

By experience it was known that children in Umeå with birch pollinosis usually started having symptoms during the first two weeks of May, had maximal symptoms during the last two weeks of May and had decreasing problems during the first weeks of June. This was believed to correlate well with the birch pollen season. The very short leafing season takes place during the most intense pollen period. Some sensitive patients started having symptoms during late April, *i.e.* during the main alder season, but as troubles depending on pollen allergy during this period were mild and infrequent they were hard to distinguish from other causes of rhinoconjunctivitis, *e.g.* upper airway infections. After the first days of June grass pollens might in­fluence symptoms. As a result of this, scoring was done from April 30 to June 10. To confirm the relevance of the chosen scoring period the 39 children in the cross-reactivity study were scored from April 16 to April 30 in 1983 with a mean daily symptom score of only 0.3 points, *i.e.* 20 children had zero scores and the remaining 19 up to 2.3 points, despite the fact that the birch pollen season this year was unusually early (Fig. 2).

![Pollens/m³(\(\vee\))](attachment:pollen-season.png)

**Fig. 2.** The pollen season in 1984 compared with the seasons 1979—1983.
It was essential for this work that the symptoms of rhinoconjunctivitis could be properly registered. The easiest way would be to ask the participating patients after each spring pollen season whether they thought that their symptoms were less pronounced, unchanged or worse compared to the season preceding therapy. This was done in the cross-reactivity study (III) and was called self-evaluation. More information could be obtained if the children were asked more frequently during the season and this was done daily with the aid of a diary. Other authors have divided the 24 hour period into day and night (Norman 1969) thus scoring twice as often, as was done in one of the present studies (V), or even into quartiles which is necessary if the circadian rhythm of the symptoms is to be studied. If the day was divided into parts then it might have been difficult to construct a true scoring for the day as the relative importance of the different parts of the day could vary among the patients and the interpretation of the scoring would thus be unclear. In addition such a complicated way for registration of symptoms was thought to diminish patient compliance, thus counteracting any possible improvement in precision.

The symptoms of rhinoconjunctivitis were divided into symptoms from the nose and from the eyes. The nose troubles could be further subdivided into dripping, blocking, itching and sneezing and the eye troubles into tear fluid, swelling, itching and congestion etc. but with the exception of one study (V) any such division was not considered to be meaningful for the present trials. Any subdivision of scoring symptoms makes it difficult to evaluate the relative importance to the patient but as the nose and the eyes are two different organs it was thought to be of interest to record symptoms separately. Thus the participating children or their parents were asked to record the symptoms from the nose and the eyes on a special form. It was also decided that a 4-grade scale would be sufficient; 0 = no symptoms, 1 = mild symptoms, 2 = moderate symptoms and 3 = severe symptoms. The points were added into a daily symptom score. To assure that the forms were properly filled in throughout the scoring period they were to be mailed after each week and if this was not done within one week the family was contacted by me.

Ideally the patients were not to take any drugs but in case of severe hayfever an "escape" medication was needed. This had to work within hours and have an effect on all symptoms of rhinoconjunctivitis. Clemastine (Tavegy®) was chosen as it was one of the best antihistamine drugs available when the studies were done (Hindmarch & Parrott 1978; Kritz 1981). If clemastine was taken it was expected to influence the symptoms and in addition the use was probably a consequence of hayfever on that day. Therefore the use of clemastine added one point to the daily symptom score.

Presence of asthma was registered in a similar way, i.e. in a 4-grade scale. If needed salbutamol (Ventoline®) could be used. This medication was not believed to influence the symptoms of hayfever and thus did not add to the daily scores.

When other medications such as nose-drops or cortisone were occasionally used the symptom scores were individually adjusted. This was done before the code was broken in the blinded studies.

The form for daily registration is given in the appendix.

One of the investigations was a collaborative study (V) in which the daytime scores were subdivided into three variables for nose symptoms and three variables for eye symptoms. In addition symptoms from the nose and the eyes during the night were noted separately giving a
maximum daily score of $8 \times 3 = 24$ points. In this paper the concomittant use of medication did not add to symptom scores.

**Provocation tests**

To evaluate the efficacy of IT it is particularly interesting to monitor the sensitivity in the shock organ with challenge tests. Nasal provocation tests are difficult to evaluate unless elaborate methods are used since nonspecific hyperreactivity may give false positive results. The test is also at best only semiquantitative (Mygind 1979).

![Parallel line assay depicting how the change in skin sensitivity is calculated](image-url)
The precision of the other relevant challenge test in patients with rhinoconjunctivitis, CPT, has not been evaluated although it has been used for decades. Therefore the test was evaluated in paper I with regard to the value as a tool to monitor IT.

When the only purpose with CPT is to demonstrate specific antibodies in the conjunctiva then a relatively high concentration of the allergen solution can be tested immediately after the initial negative control with the diluent. When the conjunctival sensitivity to an allergen preparation is to be more precisely established, however, this is done by applying successively higher concentrations to find the threshold concentration resulting in an allergic reaction. The smaller the differences between the tested concentrations, the greater is the chance of identifying small differences in the sensitivity. In this work the dilution factor between the allergen concentrations was 3.2, i.e. 100.5, which was considered to be smallest biologically meaningful difference.

Unspecific reactions are uncommon and usually easy to distinguish from allergic responses when purified allergen preparations are used. Unspecific reactions vanish more quickly than the allergic ones and do not as a rule include itching. To avoid misinterpreting unspecific reactions as positive reactions 20 minutes interval between the application of two concentrations was used and the demand for a positive CPT was both congestion and itching.

The bronchial provocation test can not replace challenge of the shock organ when monitoring patients with rhinoconjunctivitis (Stenius-Aarniala et al. 1978). In addition the test has the same drawbacks as the nasal provocation test. Futhermore the bronchial provocation test may give severe allergic reactions (Aas 1975).

Skin tests
Skin tests are used to demonstrate the presence of tissue-fixed IgE antibodies. Intradermal test are more elaborate and risky than SPT and are in addition painful which is particulary a problem in children (Indrajana et al. 1971; Hordle et al. 1984). Although not properly investigated it is possible that appropriate SPT are as precise as intradermal skin tests (Hordle et al. 1984).

In this work SPT was performed according to the Nordic guidlines (Aas & Belin 1974). To evaluate changes in the tissue-fixed antibodies as measured with SPT in the cross-reactivity study (III) a parallel line assay was used (Fig. 3; Finney 1952). The patients were tested with nine different concentrations of the allergen solution and the resulting weal areas were registered. For each patient the dose-response relationship between the allergen concentration and the resulting weal area (log values) was estimated by a straight line at each SPT. The weal area of the allergen solution was expressed in per cent of the weal area of the control histamine. If the two lines could be shown statistically to be parallel the change in skin sensitivity was estimated as the distance on the concentration axis between the lines. This was applicable to most cases. If the lines were not parallel, the distance between the two lines on the concentration axis at the level of the weal size of the histamine control was used as an estimate of the change in skin sensitivity. If, on the second SPT, only the highest concentration resulted in a weal, then the second straight line was fitted through this observation parallel to the first line. In such a case parallelity was assumed for higher concentrations than those that were actually used.

Recent investigations have shown that a modified procedure has a better precision and was easier to perform. This later method was therefore used in the OIT study (VI; Dreborg, Holgersson et al.)
Antibody analyses in vitro

As the children in this work were to be studied for several years it was necessary to decide when blood was to be drawn and how much was to be taken on each occasion. The initial idea was that in vitro changes would be obvious very soon after the start of IT, i.e. before there were any measurable in vivo changes. Therefore blood was sampled on several occasions early after the institution of IT in the cross-reactivity study. In the double-blinded OIT investigation (VI) blood was not collected as often in the beginning of IT but before and after the birch pollen season to compare the seasonal influence on the antibody levels.

Total IgE in serum was determined in duplicate using Phadebas IgE PRISTR (Pharmacia AB, Uppsala, Sweden) and specific IgE antibodies using Phadebas RASTR (Pharmacia) according to the manufacturer’s instructions. All samples in one study were run successively, i.e. within one week and with all samples from each patient on the same day, by one person with one exception: in the crossreactivity study total IgE for eight children (four matched pairs) were analyzed separately.

To calculate levels of IgG antibodies against birch in serum (IV, VI) a similar system was used (Phadebas IgG RASTR prototype, Pharmacia), comparing the samples with a reference serum. All samples from each study were analyzed during the same week by the same person and all samples from an individual patient were tested in the same run.

Antigens in a preparation can be identified with CIE (Clarke & Freeman 1966; Fig. 4). Crossed radioimmunoelectrophoresis (Weeke & Löwenstein 1973; Fig. 4) allows determination of allergens in a semiquantitative way. In the cross-reactivity study (IV) CIE and CRIE were carried out with recent modifications (Einarsson et al 1982; Uhlin & Einarsson 1983) with the aims of detecting differences between the two treatments, i.e. B and M, investigating the possibility of “new sensitivities” and relating the CRIE findings with in vivo data.

Test with polyethyleneglycols and small bowel biopsies

The abdominal side-effects that almost all children undergoing OIT recorded, were thought to be a result of a generalized inflammation of the small bowel reacting as a shock organ. The best way to study the degree of this putative inflammation would be functional and PEG absorption was chosen as a simple and reasonably well documented method (Sundqvist 1981; Fälth-Magnusson et al 1984). Samples for analysis were collected three times, i.e. before OIT, at the moment of maximum abdominal symptoms or, alternatively — if no symptoms appeared — when the highest dose of the allergen preparation was given, and finally during maintenance OIT.

In addition the morphology of the mucosa, in biopsies taken from the small intestine, was studied with scan electron microscope using a recently evaluated method (Stenling et al 1984). The biopsies were planned to be taken approximately at the same time as the PEG tests but since the two biopsies taken during ongoing OIT when the children had abdominal pain were normal the third biopsies were not taken.

Statistical methods

In clinical trials of IT for rhinoconjunctivitis due to pollinosis it is more or less obligatory to relate the symptoms with pollen counts (Aas 1982). However, the existing models for correlation of symptom scores with pollen counts (Wilkinson & Taudorf 1984) were not satisfactory. When daily symptom scores were related to the pollen counts it was obvious that the symp-
CROSSED IMMUNOELECTROPHORESIS (CIE)

Electrophoretic separation of protein mixture in agarose gel
Electrophoresis of proteins (antigens) into antibody-containing agarose gel
The formed immunoprecipitates are protein stained

CROSSED RADIO IMMUNOELECTROPHORESIS (CRIE)

Patient serum
\[ ^{125}\text{I-anti IgG or IgE} \]
Incubation 24 h with serum
Washing
Incubation 24 h with isotope
Washing
Auto radiography
x-ray film

Fig. 4. CIE/CRIE (Figure kindly provided by Roland Einarsson).

toms on a specific day were influenced not only by the pollen counts on the same day but also by the counts on preceding days. As daily pollen counts vary greatly during the season (III; V) a traditional regression analysis gave much nuisance variation. This was particularly obvious in the placebo-controlled OIT study (V), in which the treated group of children had lower scores than the placebo group on all of the 42 scored days, but still the difference between the groups was not statistically significant due to the big individual variations. It was also obvious that pollen counts had to be transformed and several transformations were tried before a square root transformation was chosen. The time-series analysis employed to overcome the problem of swiftly varying pollen counts is presented in one of the papers (II).
With the method used the daily symptom score can vary from 0 to 7 points. For every day there is an accumulation of children scoring 0 points and on days with high pollen counts there is also an accumulation of children scoring 7 points as they can neither have lower nor have higher scores. This means that during years with very low or very high pollen counts it is more difficult to find true differences between groups of patients than during years with average counts. When daily scores were added to a total score for the season there was much less accumulation seen at the “ends” of the scale and parametric statistical methods could be used, although non-parametric methods used for controlling the results gave almost the identical results. However, when the change in total symptom scores from one year to another was compared between the children and related to other variables a skewness was found and in one study a transformation of the total scores was used (IV). Also the values obtained for allergen extract potencies and of levels of antibodies were logarithmic transformed owing to skewness.

The CPT study resulted in the possibility of constructing a power function presented in detail (I).
RESULTS AND DISCUSSION

The two methodological investigations, i.e. the CPT study (I) and the statistical paper (II), were necessary for evaluation of the findings in the cross-reactivity and the OIT studies. Some the conclusions from the CPT study, i.e. that the method is simple and safe, were confirmed by the later IT studies. The statistical method has recently been used successfully in an unpublished study (Sobocki et al).

The finding in the cross-reactivity study that IT with the mixture was slightly better than treatment with the birch pollen preparation was astonishing, especially as no hazel pollens are found in Umeå and as the difference between the two treated groups was most pronounced during the main birch season and not immediately after the alder season. The M group had probably not received higher doses than B, even though the actual strength of the mixture was unknown, as the preparation was made by mixing three pollen preparations in equal amounts (in BU) and the potency of the mixture was said to be the sum of the potencies for the included preparations in BU. Although the difference between the groups as assessed with symptom scores was statistically clear-cut it is possible that it was spurious to at least some degree. Therefore the conclusion is that IT with M is at least as good as IT with B.

According to the CPT study a difference in threshold concentration of factor ten ($10^1$) is needed to detect a true difference with a probability of 95% if there are 19 patients in each group. To reveal a difference of factor 3.2 with a 95% probability 40 patients are needed in each group. The sensitivity of CPT thus appears to be too low for an investigation planned as the cross-reactivity study. Although not studied it is reasonable to assume that the sensitivity of SPT is not superior to that of CPT. Thus daily symptom scoring performed as in this work may be the most sensitive method for evaluation of IT efficacy.

The difference in symptom score between the groups was statistically highly significant but clinically probably only of minor importance. All but one of the children improved irrespective of the preparation given. The patient who did not improve was the only one without any reactions during rush IT. He also had the lowest level of IgE antibodies against birch of all participating children and his lymphocytes were not stimulated by the birch preparation (Möller et al 1984). Although he fulfilled the criteria for inclusion he apparently had only a low degree of birch pollen allergy.

Several immunochemical data before IT or during the first months of therapy correlated to the improvement in the B group but not in the M group. The clinical relevance of these correlations and the differences between the groups is obscure and possibly spurious. In the M group it was not possible to predict the outcome of IT with any of the in vivo or in vitro data prior to treatment or with a combination of several parameters. Several authors have pointed out that there are only poor correlations between in vitro and in vivo data during immunotherapy (Lichtenstein et al 1968; Norman 1978; Grimmer 1980; Rocklin 1983). The clinical relevance of the statistically clear-cut difference between the two treatment groups in symptom scores is also dubious.
During the pollen season 1983, i.e. 3–6 months after the cessation of IT, the symptom scores were higher than during 1982 in both patient groups. The obvious reason for this increase of symptoms is the extremely high pollen counts in 1983 but it may also be due to a decreasing effect of IT. During 1984 only 38 children were scored as one child had left the country. Another three children had left Umeå. The mean total score was 58 points with very little difference between the two treated groups. The pollen season 1984 took place early but the intensity was similar to 1981 and 1982 when the children had less symptoms (Fig. 2). This indicates that the efficacy of IT does not last for all patients. Long term studies are needed to evaluate whether IT has a lasting effect or not.

From a theoretical point of view the obvious disadvantage when treating with the mixture instead of the pure pollen preparation would be that more allergens are injected, some of which the patient is not allergic to. Despite some earlier investigations supporting this possibility several recent Nordic investigations show that the development of “new sensitivities” as a result of IT for allergy against grass pollens, mites and animal danders is uncommon among adults (Österballe et al 1982; Grimmer 1980; Einarsson et al 1983; Nordvall et al 1984; Löwenstein et al 1984; Maasch et al 1985; Ipsen, Löwenstein et al 1985). In contrast to this we found that the development of “new sensitivities” was common in children receiving IT. A possible explanation for this difference would be that the children had not had the time to develop IgE antibodies prior to the treatment against all allergens that they according to their genetic code had the capacity to develop sensitivity to. It is thus possible that they would have developed these sensitivities in due time even if not given IT. The development of “new sensitivities” did not, however, impair the treatment results as measured with symptom scores.

The immune system of the rabbit can identify at least 25 different antigens in birch pollen but only eight of these are allergenic in man, two of them dominating (IV). The children developed IgG antibodies against only six of the potential antigens. The reason for the rabbit being more reactive may be genetically determined but another possible explanation is that the animals were sensitized parenterally with high doses of the allergens and with Freund’s complete adjuvant (Belin & Strannegård 1971; Marsh 1975; Nordvall et al 1982; Björkstén & Ahlstedt 1984). What makes some of the antigens more allergenic than others is unknown but it may reflect a general antigenicity since the more common the antigen is in the birch pollen allergen preparation the greater is the chance that the human immune system will develop antibodies against that antigen (Dreborg, Einarsson & Longbottom). Thus there would be no structural differences in the allergenic molecules from birch making them more or less allergenic than other protein antigens of the same molecular size that are not allergenic. However, an interesting observation is that a birch pollen allergen preparation can partly inhibit immunochemical reactions with antigen M from cod, in contrast to allergens from other sources (Elsayed et al 1980). Antigen M is one of the most well-studied allergens and the part of the protein chain necessary for the allergenic events is defined. There is a possibility that a similar structure is found among the allergenic proteins of birch even though cod and birch do not appear to cross-react. Thus the capability to induce IgE response may possibly be a consequence of molecular structure per se (Aas 1982; Nordvall et al 1982; Nordvall et al 1985).

The first study of OIT was mainly planned as an open pilot study although a part of it was intended as a double-blind trial. It was, however, neither difficult for the responsible investigator (CM) nor for the children with active treatment to guess to which treatment group they belonged. Thus no part of the study was truly controlled. Despite this an efficacy was indicated not only by in vivo data, i.e. symptom score and CPT, but also by in vitro data, i.e. birch-specific IgE and IgG. The pronounced abdominal side-effects were unexpected and to avoid them a much more cautious dose schedule was necessary.
The second and truly controlled study of OIT showed that this mode of IT had a clinical efficacy as measured with symptom scores. Also the SPT results indicated an efficacy. On the other hand the treated children had changed their conjunctival sensitivity very little after ten months of treatment. Several explanations for this are possible (V).

The extremely pollen-rich season 1983 probably blunted the in vivo results as discussed above. Another factor that would be expected to have diminished the difference in symptom scores between the treated and the untreated groups of children is that OIT was given only for three months. In the cross-reactivity study it took 1½ years to demonstrate good treatment efficacy (perhaps partly owing to the fact that the pollen season 1979 — before IT — was unusually mild). The problem with the unusually severe pollen season 1983 and symptom scores could be overcome with the time-series analysis which showed a statistically significant difference between the actively-treated and the placebo-treated children.

The levels of IgE antibodies against birch in the individual actively-treated children either were not changed or were at least doubled as expressed in PRU. In other words there appeared to be an either-or situation. For IgG against birch the increases of antibody levels were more moderate among the children who responded. The increase in IgE antibody levels during the first months of OIT was similar to the findings in patients undergoing SIT (IV; Foucard & Johansson 1976; Kjellman et al 1976; Norman 1978; Rocklin 1983) and was therefore taken as an indication that OIT was effective. There was no seasonal increase in levels of IgE against birch in the actively treated children in contrast to the controls, in most of whom increases were seen. This lack of seasonal IgE increase also indicated that OIT was effective (Sadan et al 1969; Levy et al 1971; Reisman et al 1975; Pence et al 1976).

To avoid the embarrassing side-effects the dose schedule was modified, i.e. the doses were increased only slowly. The children were also instructed to reduce the dose immediately if side-effects were suspected. As a result of these measures only three of the 14 treated children thought that the side-effects were a problem at all.

Since the side-effects for the treated children in most cases were minimal and six of the untreated children had similar problems, i.e. an unspecified abdominal discomfort, it was not possible for the investigator or for the patients to tell whether active treatment was given or not. Indeed most of the children thought that they belonged to the placebo group as they had more symptoms of rhinoconjunctivitis during the extremely intense pollen season 1983 than during 1982. During the first three months of treatment, i.e. during the spring 1983 before the pollen season, I tried on two separate occasions to guess how the children were treated with a correct conjecture for approximately 50% of the children.

In the total group of 30 children, 5 actively-treated and 10 placebo-treated children reported that it was difficult to remember when to start taking the capsules for seven consecutive days. After the double-blinded part of the trial was finished the children in the control group were given active capsules once a week as done for the patients in the pilot study treated in Stockholm (III) and this turned out to be much easier to remember.

The dose schedule used in the double-blinded study appeared to be safe since no generalized reactions occurred, no small bowel changes were detected with PEG absorption measurements or with biopsies and the safety data were within expected limits. It is, however, unlikely that the small bowel was not affected at all by the allergen preparation. Possibly neither measurements of PEG recovery nor biopsies of the proximal part of the small intestine in only two children could reveal moderate or localized changes.
The statistically clear-cut PEG absorption changes among untreated control patients in the beginning of the pollen season were unexpected. The clinical relevance of PEG recovery changes is not clear. It would appear that untreated children with birch pollinosis already have changes in intestinal permeability as assessed with a mixture of differently sized PEG after a few days of the spring pollen season and that children undergoing OIT are protected from these changes. Studies of possible seasonal gastrointestinal disturbancies in untreated and treated pollen allergic patients are warranted.

The main drawback with OIT is that patient surveillance may diminish, since there is no need for regular visits to the doctor's practice. Owing to misunderstanding the doses may be too low or too high, particularly if the treatment is introduced in routine clinical practice. Beside the decreased monitoring of the IT the opportunities for the patient to discuss other problems, e.g. symptomatic medication, will be fewer. These drawbacks may, however, be easily overcome, provided the responsible physician is aware of the needs of the patients. It must also be pointed out that so far OIT has been successful only with a birch pollen allergen preparation.
SUMMARY OF RESULTS

I. Conjunctival provocation tests using a birch pollen allergen preparation were 92% reproducible within an allergen strength difference of one 10-potency. A power function made it possible to determine the number of patients needed to discriminate CPT sensitivity of a given magnitude between two populations. A consequence of repeated CPT may be an increase in levels of serum-IgE against the allergen preparation. The test is useful in clinical routine and in research being easy to perform, safe and with a good precision.

II. A dynamic time series model for comparison of treatments of allergy, allowing for adjustment to daily pollen counts, was proposed and applied to two clinical trials. The difficulties with a more traditional regression approach seemed possible to overcome with the dynamic model.

III and IV. During three years of subcutaneous immunotherapy children treated with a pollen allergen preparation made from a mixture of birch, alder and hazel reduced their symptom scores at least as much as children treated with a preparation made from birch only. There were no differences between the groups regarding subjective improvement, conjunctival sensitivity, skin sensitivity, changes in levels of total IgE, IgE against birch, alder and oak or IgG antibodies against birch. Nor were there any differences in the patterns of crossed radioimmunoelectrophoresis to birch (IgE and IgG) or to oak (IgE). Immunochemical findings before and during the first months of immunotherapy correlated with improvement as measured by symptom scores in the birch group but not in the mixture group. Findings of "new sensitivities" in IgE-CRIE after immunotherapy were common in children below 13 years.

V. Oral immunotherapy using high doses of a birch pollen allergen preparation in enteric-coated capsules appeared to be effective as indicated by lower symptom scores, reduced conjunctival sensitivity and increased levels of IgE antibodies against birch. However, gastrointestinal side-effects were pronounced.

VI. In a truly double-blind study of oral immunotherapy with high doses of birch pollen allergen in enteric coated capsules using a different dose schedule than in the previous study it was shown that the actively-treated children had lower symptom scores than the placebo treated children during the birch pollen season already after three months of treatment. After ten months of therapy the actively-treated group of children had reduced their skin sensitivity against birch more than the placebo group. Compared to the placebo group the actively-treated children more often increased their levels of IgE and IgG antibodies against birch before the season but more seldom increased their levels of IgE against birch during the season. Side effects were not embarrassing.

VII. In the double-blinded study of oral immunotherapy it was shown that actively-treated children did not change their intestinal permeability characteristics as assessed with urinary recovery of differently sized polyethyleneglycols. However, in the placebo-treated children the recovery of larger molecules was decreased in the beginning of the pollen season. From two additional children, openly given oral immunotherapy, small bowel biopsies taken showed no morphological changes.
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APPENDIX

Diary for one week used during the birch pollen season.

SYMPTOMKORT FÖR BJÖRKALLERGIKER 1983

Namn:  Födelsedatum:

I rutorna för Tavegyl anger man hur många tabletter man tagit under dagen.
I rutorna för annan medicin anger man medicinens namn samt hur mycket man tagit.

Allergiska besvär (från ögon, näsa eller luftvägar) graderas: 0 = inga besvär
1 = lättta besvär
2 = måttliga besvär
3 = svåra besvär

Allergiska besvär från luftvägarna kan till exempel vara hosta, tungt att andas eller pip i bröstet.
Andra symptom kan till exempel vara trötthet, illamående, ont i magen, huvudvärk, utslag eller feber.

<table>
<thead>
<tr>
<th>Datum</th>
<th>Tavegyl</th>
<th>Annan medicin</th>
<th>Besvär från ögon</th>
<th>Besvär från näsa</th>
<th>Besvär från luftvägar</th>
<th>Andra symptom (beskriv kortfattat)</th>
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Det ifyllda formuläret skickas så snart som möjligt i bifogade svarkuvert till:

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Barnkliniken
901 85 Umeå

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