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Natural Rubber Latex
Allergy in Children

ACADEMIC DISSERTATION
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Contents

List of original publications

The present thesis is based on the following original papers referred to in the text by the Roman numerals I-V.

I.   Ylitalo L, Turjanmaa K, Palosuo T, Reunala T. Natural rubber latex allergy in children who had not undergone surgery and children who had undergone multiple operations. J Allergy Clin Immunol 1997;100:606-12.

II.   Ylitalo L, Alenius H, Turjanmaa K, Palosuo T, Reunala T. IgE antibodies to prohevein, hevein, and rubber elongation factor in children with latex allergy. J Allergy Clin Immunol 1998;102:659-64.

III.   Seppälä U, Ylitalo L, Reunala T, Turjanmaa K, Kalkkinen N, Palosuo T. IgE-reactivity to patatin-like latex allergen, Hev b 7, and to patatin of potato tuber, Sol t 1, in adults and children allergic to natural rubber latex. Allergy (in press)

IV.   Ylitalo L, Mäkinen-Kiljunen S, Turjanmaa K, Palosuo T, Reunala T. Cow's milk casein, a hidden allergen in natural rubber latex gloves. J Allergy Clin Immunol 1999; 104:177-80.

V.   Ylitalo L, Alenius H, Turjanmaa K, Palosuo T, Reunala T. Natural rubber latex allergy in children; a follow-up study. (submitted for publication)

Some unpublished results are also presented in this thesis and they are indicated by the abbreviation UR.

Abbreviations

ELISA enzyme-linked immunosorbent assay

IgE   immunoglobulin E

HPLC high-performance liquid chromatography

kD   kilodalton

mM   millimolar

NRL   natural rubber latex

OD   optical density

PBS   phosphate-buffered saline

RAST radioallergosorbent test

REF   rubber elongation factor

RIE   rocket immunoelectrophoresis

RRIE   rocket radioimmunoelectrophoresis

SD   standard deviation

SDS   sodium dodecyl sulfate

SPT   skin prick test

A. INTRODUCTION

During the last twenty years, natural rubber latex (NRL) allergy has become a well-known medical problem because of the increasing frequency of NRL-induced reactions. Health care workers have been recognised as the major occupational risk group for NRL allergy. Other high-risk groups include children with spina bifida and other children requiring multiple surgical operations, atopic individuals, housekeeping personnel and other glove-wearing persons (Turjanmaa et al. 1996, Warshaw 1998). The frequency of NRL allergy in health care workers ranges from 2.9% to 17% (Turjanmaa 1987, Yassin et al. 1994). In children with spina bifida the frequency is much higher, ranging from 23% to 65% (Yassin et al. 1992, De Swert et al. 1997). In contrast to NRL-allergic children with spina bifida, only a few reports of NRL allergy in children without a history of multiple operations have been published in recent years (Shield and Blaiss 1992, Akasawa et al. 1993, Sorva et al. 1995, Liebke et al. 1996).

In children and adults the symptoms of IgE-mediated NRL allergy vary from contact urticaria to asthma and anaphylaxis (Turjanmaa et al. 1996, Warshaw 1998). Severe systemic reactions have been reported to occur, usually during operations and especially in children with spina bifida (Meeropol et al. 1993, Kelly et al. 1994). There is also one report describing anaphylaxis in two non-operated children from rubber balloons (Axelsson et al. 1988).

Clinical history as well as clinical and laboratory tests are the basis for the diagnosis of NRL allergy. Because the symptoms of NRL allergy are not always characteristic and may even be absent (Yassin et al. 1992, Nieto et al. 1996), *in vivo* and *in vitro* tests are necessary to confirm the diagnosis. The skin prick test (SPT) has been used for several years to screen and diagnose NRL-allergic patients, and with standardized techniques and allergens this test has proved to be sensitive and also specific in adult patients (Turjanmaa et al. 1997, Blanco et al. 1998). IgE antibodies to NRL can be measured by the radioallergosorbent test (RAST) or by the AlaSTAT method, but the sensitivities and specificities of these tests seem to be inferior to the SPT method (Mäkinen-Kiljunen and Turjanmaa 1995, Blanco et al. 1998). A challenge test with NRL glove (use test) has been recommended for confirmation of the NRL allergy diagnosis if there is a discrepancy between the other NRL tests and the clinical history (Turjanmaa et al. 1995, Warshaw 1998). An inhalation challenge test can be performed to confirm the diagnosis of NRL-induced asthma (Vandenplas et al. 1995).

NRL from the rubber tree *Hevea brasiliensis* contains more than 200 polypeptides of which several have been identified as binding IgE from NRL-allergic patient sera (Alenius et al. 1994c). Much research has been performed to identify the major and clinically significant NRL allergens. Prohevein (Hev b 6.01), hevein (Hev b 6.02) and Hev b 5 seem to be the major allergens in NRL-allergic adults and rubber elongation factor (Hev b 1) in children with spina bifida (Alenius et al. 1995a, 1996a,c, Akasawa et al. 1996, Slater et al. 1996, Yeang et al. 1996, Chen et al. 1997a).

Many patients with NRL allergy have been reported to be sensitized also to a variety of plant foods such as banana, avocado, kiwi fruit, chestnut and potato (Blanco et al. 1994,

Beezhold et al. 1996). This so-called "latex-fruit syndrome" has been suggested to be based on cross-reactivity between NRL and various food allergens. Cross-reacting allergens in NRL and fruits have been demonstrated by *in vitro* inhibition assays (Lavaud et al. 1997), but less information is available on the nature of the cross-reacting allergens.

The purpose of the present study was to examine the frequency of NRL allergy in children admitted for evaluation of inhalant and food allergies. The diagnosis of NRL allergy was based on strict criteria using three tests (SPT, latex RAST and glove use test). To compare clinical findings, SPT and latex RAST results, the NRL-allergic children found in screening were divided into two groups. The first group included children who had no history of operations and the second group children with spina bifida and other children with a history of multiple operations. In addition, the frequency of IgE antibodies to purified NRL allergens (Hev b 1, Hev b 6.01, Hev b 6.02, Hev b 5) was determined in these two groups of NRL-allergic children. The aim of a follow-up study of the NRL-allergic children was to examine the clinical outcome and possible changes in SPT reactivity and IgE antibody levels to NRL allergens. The SPT reactivity to several fruits and potato was also examined and an *in vitro* study was performed to determine whether NRL-allergic children have IgE antibodies to a patatin-like NRL allergen, Hev b 7. False positive NRL glove use tests with casein-containing glove were encountered in 5 cow´s milk-allergic children and a study was performed to examine whether other NRL glove brands contained casein.

B. REVIEW OF THE LITERATURE

1. History of NRL allergy

There are two types of rubber allergy; delayed type IV allergy to chemicals added to NRL during manufacture and immediate type I allergy to NRL proteins. The first case of immediate NRL allergy was reported in 1927 by Stern who described a patient with severe generalized urticaria caused by a rubber dental prosthesis (Stern 1927). The modern history of NRL allergy began over 50 years later when Nutter described contact urticaria from household gloves in a housewife (Nutter 1979). Förström (1980) reported a nurse with contact urticaria from surgical gloves and with this case focused attention on the occupational nature of NRL allergy. Köpman and Hannuksela (1983) were the first to show by the Prausnitz-Küstner passive transfer test that NRL allergy is IgE-mediated. This was later confirmed by several researchers (Frosch et al. 1986, Axelsson et al. 1987, Warpinski et al. 1991). The main symptom described in the first reports was contact urticaria, but rhinitis and asthma after opening a sterile surgical glove bag were also reported as symptoms of NRL allergy (Carrillo et al. 1986). A few years later, glove powder was confirmed to be contaminated with NRL allergens (Turjanmaa et al. 1990). The first case of anaphylaxis during a surgical operation was reported by Turjanmaa et al. (1984). Thereafter, several anaphylactic reactions were recorded during operations and especially during barium enema examinations that contain NRL balloons. These examinations also caused several deaths (Leynadier et al. 1989, Ownby et al. 1991, Dillard et al. 1992).

During the last 10 years, NRL allergy research has, besides clinical research, concentrated on the identification and characterization of NRL allergens. It has been demonstrated by the two-dimensional immunoblot method that NRL contains approximately 240 polypeptides, of which 57 bind IgE from the sera of NRL-allergic patients (Alenius et al. 1994c). Several NRL allergens with molecular weights of 14, 20, 27, 30, 46, 54 and 75 kD have been identified by immunoblot studies (Alenius et al. 1991, 1993, 1994a, Alenius 1995, Jäger et al. 1992, Slater and Chhabra 1992, Beezhold et al. 1994, Akasawa et al. 1995). Rubber elongation factor (Hev b 1) was suggested to be a major NRL allergen and the only allergen present in one brand of NRL surgical glove (Czuppon et al. 1993). Later on, Hev b 1 was confirmed to be a significant allergen in children with spina bifida and other children with a history of multiple operations (Alenius et al. 1996a, Yeang 1996, Chen et al. 1997b). In 1995, prohevein, Hev b 6.01, was shown to be a major allergen in NRL-allergic patients (Alenius et al. 1995a). As early as 1988, a small molecular weight peptide eluting from NRL glove extract was reported to be capable of causing positive SPT reactions in NRL-allergic patients (Turjanmaa 1988). In 1996, a 4.7 kD peptide in NRL, hevein (Hev b 6.02), was shown to be a major NRL allergen and the main IgE binding domain in prohevein (Alenius et al 1996c, Chen et al. 1997a). In 1996, both Akasawa (1996) and Slater (1996) identified an acidic protein, Hev b 5, and reported that it was also a major IgE binding allergen in NRL. Until now, 9 NRL allergens have been characterized in more detail (Breiteneder and Scheiner 1998, Posch et al. 1998).

The first report of established cross-reactivity between NRL and banana was in 1991 (M'Raihi et al. 1991). Thereafter, several reports on NRL allergy and fruit cross-reactivity have been published (Blanco et al. 1994, Mäkinen-Kiljunen 1994, Beezhold et al. 1996). The cross-reactivity of NRL and fruit allergens, the so-called "latex-fruit syndrome", is now well established clinically and much research is going on to discover the molecular mechanisms of this cross-reactivity (Mikkola et al. 1998, Diaz-Perales et al. 1998, Chen et al. 1998).

The history of NRL allergy in children started as early as 1988 when Axelsson et al. (1988) described three atopic children who developed anaphylaxis and angioedema after exposure to rubber balloons. The following year Slater (1989) described two children with spina bifida who experienced anaphylaxis during operation, and suggested that the allergy was due to an IgE-mediated reaction to NRL. In 1991, Slater et al. (1991) measured IgE antibodies to NRL with RAST and showed that 34% of the children with spina bifida had these antibodies. Soon after that several reports of NRL allergy in children with spina bifida and other children requiring multiple operations were published and in many reports serious symptoms, like anaphylactic reactions, were described (Gold et al. 1991, Nguyen et al. 1991, Meeropol et al. 1993, Kelly et al. 1993, 1994, Beaudouin et al. 1994, Kwittken et al.1995). In the latter half of the 1990s many studies of the risk factors of NRL allergy in children with spina bifida have been conducted which show that the main risk factors are the number of surgical operations performed and atopic diathesis of the patients (Nieto et al. 1996, Michael et al. 1996, Mazon et al. 1997). In contrast to children with spina bifida and other multioperated NRL-allergic children, only a few reports exist on non-operated children. These few reports suggest that atopic disorders predispose also non-operated children to NRL allergy (Shield and Blaiss 1992, Akasawa et al. 1993, Sorva et al 1995, Liebke et al. 1996).

2. Symptoms of NRL allergy

The most frequently reported symptom of NRL allergy is contact urticaria (Table 1). The onset of contact urticaria usually occurs from 5 min to 1 h after contact with NRL products. Without treatment the symptoms disappear in 30 min to 2 hours after the exposure. The additional symptoms of NRL allergy fit well with the so-called contact urticaria syndrome (Maibach and Johnson, 1975). This includes 4 clinical stages: (1) localized urticaria on the contact area, (2) generalized urticaria with angioedema, (3) urticaria with asthma, rhinoconjunctivitis and orolaryngeal and gastrointestinal symptoms and (4) urticaria with anaphylactic reaction. After contact urticaria, rhinoconjunctivitis caused with certainty by airborne NRL allergens is the next most common NRL allergy symptom, followed by asthma (Table 1, Turjanmaa et al. 1995, Jäger et al. 1992, Heese et al. 1994, Tarlo et al. 1994, Vandenplas et al. 1995). Angioedema is not infrequent and may arise on mucosal contact, e.g., after oral, vaginal or rectal exposure to NRL products (Turjanmaa et al. 1996). In addition to immediate type I allergy symptoms, NRL allergy can manifest on the hands of glove users as protein contact dermatitis or allergic contact dermatitis (Turjanmaa 1988, Heese et al. 1994, Janssens et al. 1995, Wilkinson and Burd 1998). However, Wakelin et al. (1999) patch tested 608 patients with NRL, and did not find any patients with positive type IV allergic patch test reactions showing that allergic contact dermatitis to NRL is very uncommon. Turjanmaa and Reunala (1989a) showed that a few immediate type NRL

allergic patients with hand eczema can have concomitant type IV allergy to rubber chemicals.

Table 1. Frequency of symptoms in NRL-allergic adults and children.

Symptom	Adults*	Children with spina bifida
Contact urticaria	72% - 79%	69%¤
Rhinitis	13% - 16%	92% ¤ (both rhinitis
Conjunctivitis	16% - 28%	and conjunctivitis)
Asthma	2% - 4%	+ §
Anaphylaxis	7% - 10%	+ §

* health care workers and patients from other occupations (Turjanmaa et al. 1995)
¤ questionnaire-based study (Pearson et al. 1994)
§ described in several case reports, frequency unknown

Severe systemic reactions are frequently reported in NRL-allergic children with spina bifida (Slater 1989, Gold et al. 1991, Meeropol et al. 1993, Kelly et al. 1994). These children are exposed during operations to NRL gloves and other medical rubber products mainly through mucous membranes due to which allergen penetration seems to occur more easily than through an intact skin. Kwittken et al. (1995) studied symptoms in 35 NRL-allergic children, most of them with a history of multiple operations, and found that almost half of the children had experienced an anaphylactic reaction perioperatively while the others had sustained milder symptoms. Pearson et al. (1994) made a questionnaire-based study of 110 children with spina bifida and detected 13 NRL-allergic children. The symptoms were rhinitis/conjunctivitis in 92%, angioedema in 83%, urticaria in 69%, sneezing in 67%, dyspnea in 54%, wheezing in 23% and/or dizziness in 17% of the children.

The main cause of symptoms in exposed adults is NRL gloves (Turjanmaa et al. 1995). In addition, several kinds of medical and non-medical rubber products, such as catheters, dental dams, rubber bands and condoms, may elicit allergy symptoms. In one study of NRL-allergic children with spina bifida, balloons (92%) and gloves (69%) were the most common cause of the symptoms (Pearson et al. 1994). Exposure to pacifiers, teats and balloons has also been described as causing NRL allergy symptoms, even anaphylactic reactions (Axelsson et al. 1988, Mäkinen-Kiljunen et al. 1992a).

3. Diagnosis of NRL allergy

A detailed history of allergy symptoms and clinical examination are always important when diagnosing NRL allergy. It should be noted, however, that patients may have only mild symptoms which easily pass unnoticed or may be confused with other allergies. In addition, patients sensitized to NRL can also remain asymptomatic (Yassin et al. 1992, Hadjiliadis et al. 1995, Sussman and Beezhold 1995, Nieto et al. 1996). Therefore, the diagnosis of NRL allergy should not be based only on a positive history but also on *in vivo* and *in vitro* allergy tests. It should also be noted that at present there are no diagnostic test methods for NRL allergy which are 100% sensitive and specific.

3.1. Skin prick test

Many previous studies have used SPT in diagnosing NRL allergy (Wrangsjö et al. 1988, Moneret-Vautrin et al. 1993, Turjanmaa et al. 1995, Hadjiliadis et al. 1995, De Swert et

al. 1997, Blanco et al. 1998). Because of the lack of standardized commercial NRL allergens, test materials such as NRL glove extracts and crude NRL with or without ammonia have been used in skin prick testing. Extracts made from a high-allergenic NRL glove brands (Triflex[R], Baxter, Valencia, CA, USA) have been used by us for many years for diagnostic purposes and it has proved to be a good and safe test material, both in the NRL-allergic children and adults (Turjanmaa et al. 1995). At present, a standardized, commercial SPT reagent (Stallergènes, Antony, France) is available on the European market and this reagent has a 93% sensitivity and 100% specificity when testing NRL-allergic patients (Turjanmaa et al. 1997). A few other non-standardized commercial SPT reagents are also available in Europe (ALK a/s, Hørsholm, Denmark) and in Canada (Bencard, Mississuaga, Ontario, Canada). The sensitivities and specificities of the different SPT reagents are given in Table 2. Recently, Hamilton et al. (1998) evaluated a new SPT reagent from the Greer Laboratories (Greer NAL reagent, Lenoir, NC). It showed a 95% sensitivity and 100% specificity when tested in 134 NRL-allergic and 190 non-allergic patients. Mild systemic adverse reactions were recorded in 16% of the patients tested and this reagent waits for approval from the Food and Drug Administration (FDA).

Table 2. Sensitivities and specificities of different SPT reagents.

SPT reagent	Sensitivity Adults# / Children with spina bifida *	Specificity Adults# / children with spina bifida*
Glove extract¤	84%[3]-92%[1] / 100%	100%[1,3] / 95%
Stallergènes	88%[1]-93%[2] / 64%	100%[2,3] / 91%
ALK	54%[1]-90%[3] / n.k	100%[3] / n.k
Bencard	92%[1] / n.k	100%[1] / n.k

\# Turjanmaa et al. 1994[1], 1997[2], Blanco et al. 1998[3]
* De Swert et al. 1997
¤ Triflex® glove
n.k = not known

Skin prick testing has been used safely for years by us and other researchers in the screening and diagnosis of NRL allergy (Turjanmaa et al. 1996, Hadjiliadis et al. 1995). Some researchers have reported adverse reactions and even anaphylaxis from skin prick testing (Bonnekoh et al. 1992, Kelly et al. 1993). The differences recorded in the frequency of adverse reactions seem to be due to variable test techniques and allergen materials. Prick test technique is regarded safer than intradermal testing and one peaked SPT lancet with shoulders can be recommended over multipeaked lancet (Turjanmaa et al. 1995). If there is still a need to use in-house SPT reagents, the allergen concentration of the NRL source material should be known in order to avoid adverse reactions or as well as false negative test results.

3.2. Latex RAST and other tests measuring IgE antibodies

IgE antibodies to NRL in the patient's serum have been most often measured by the CAP RAST method (Pharmacia, Uppsala, Sweden). Different studies have given varying results for the sensitivity and specificity of the RAST method (Table 3). Latex RAST seems to detect well highly allergic patients, but this test has been negative even in NRL-allergic patients with anaphylactic reactions (Axelsson et al. 1988, Leynadier and Dry 1991, Jäger et al. 1992). Moreover, the sensitivity and specificity of latex

RAST have not been clarified in the NRL-allergic children who have no history of multiple operations (Sorva et al. 1995). A second serologic method, AlaSTAT (Diagnostic Products Corporation, Los Angeles, CA, USA), is less frequently used in Europe, but recent studies have showed a good agreement between the results obtained with latex RAST and AlaSTAT methods (Blanco et al. 1998, Hamilton et al. 1999).

Table 3. Sensitivity and specificity of latex CAP RAST and AlaSTAT in NRL-allergic patients.

Authors	Patients/ controls (n)	Criteria for NRL allergy diagnosis	Sensitivity CAP / AlaSTAT	Specificity CAP / AlaSTAT
Mäkinen-Kiljunen and Turjanmaa 1995	61 / 70 adults and children	Positive SPT and/or glove challenge	77% / -	70% / -
De Swert et al. 1997	17 / 57 children with spina bifida	Positive glove challenge	89% / -	93% / -
Blanco et al. 1998	50 / 50 adults	Positive clinical history and SPT	86% / 84%	<70% / <70%
Hamilton et al. 1999	131/181 adults	Positive clinical history and SPT	76% / 73%	97% / 97%

IgE antibodies to NRL can also be measured by the ELISA method. In one study, which included also patients with spina bifida, in-house ELISA method showed a 87% sensitivity and 66% specificity (Kelly et al. 1993). Semiquantitative assays, which have been used to detect IgE binding to NRL allergens, include immunoblotting, and other immunoelectrophoretic methods. (Mäkinen-Kiljunen et al. 1992b, Alenius et al. 1993, 1994a). Basophil histamine release test has also been evaluated in the diagnosis of NRL allergy with good results (Turjanmaa et al. 1989a).

In the latex RAST, AlaSTAT and other methods mentioned above, crude NRL or uncharacterized NRL have been used as allergens. At present, it is not known whether crude NRL contains all relevant allergens and if their concentrations are appropriate for diagnostic purposes. There is some evidence that the manufacture of gloves may change the NRL allergens or even produce neo-allergens (Mäkinen–Kiljunen et al. 1992b). On the other hand, crude NRL is now known to contain cross-reactive allergens such as profilin, which could be one cause for false positive reactions in the latex RAST and AlaSTAT (Vallier et al. 1995, Blanco et al. 1998).

3.3.   Challenge tests

3.3.1   Use test

A use test with a NRL glove is needed to confirm the NRL allergy diagnosis when there is discrepancy between clinical history and the SPT and/or latex RAST results (Turjanmaa et al. 1995). It is not recommended for patients with a history of NRL-related anaphylaxis when SPT and/or RAST are positive. The NRL glove use test should be performed with a highly allergenic glove brand (Turjanmaa et al. 1996, Baur et al. 1998). It should always be started with a finger piece of a glove because the use test with the whole glove on eczematous skin can cause anaphylaxis (Turjanmaa and

Reunala 1988). If no wheals occur with a finger piece, the test is continued with a whole glove. Both tests are performed on a wetted skin in order to get efficient allergen elution and penetration (Turjanmaa et al.1995, De Swert et al. 1997). A vinyl glove is used as a negative control to exclude dermographism. If the 15-30 min whole NRL glove use test remains negative, a prolonged use test for 5-7 days can be performed in order to exclude mild NRL allergy or protein contact dermatitis. To increase allergen penetration into the skin from the NRL gloves during challenge, Hamilton et al. (1997) first punctured the skin of the hands with needle, and then applied the NRL gloves.

3.3.2 Inhalation tests

When rhinitis or asthma is suspected to be caused by NRL products, an inhalation challenge test can be performed to confirm the diagnosis. Bronchial provocation tests have been performed by inhaling nebulized glove extract or by handling and shaking powdered NRL gloves in a special challenge room. Spirometric measurements were done before and after these challenges (Jäger et al. 1992, Pisati et al. 1994, Vandenplas et al. 1995). A nasal provocation test has been performed by applying NRL glove powder on a cotton swab to the nasal mucosa for 5 min. Allergic response is detected by anterior rhinoscopy, rhinomanometry and measurement of nasal secretions (Kujala et al. 1995).

4. Frequency and risk factors for NRL allergy

NRL allergy affects people who are frequently exposed to gloves and other NRL products. The increasing number of reports of NRL allergy published in recent years suggest that the incidence is still increasing. One reason for this seems to be the outbreak of HIV infection in the 1980s with the subsequent increase in the use of NRL and other protective gloves in the health care services. On the other hand, awareness of and improved diagnostic facilities for NRL allergy may be other reasons for the increased reporting of NRL allergy.

The present frequency of NRL allergy in the general population is not known accurately. The prevalence seems, however, to be less than 1% (Table 4, Moneret-Vautrin et al. 1993, Turjanmaa et al. 1995). SPT screening studies in adults suspected of or sustaining atopic disorders have shown NRL allergy frequencies from 0.85% to 9.4% (Moneret-Vautrin et al. 1993, Hadjiliadis et al. 1995, Turjanmaa et al. 1995). One serologic screening study using the AlaSTAT method disclosed a prevalence as high as 6.4% in blood donors in the USA (Ownby et al. 1996). A corresponding study in Italian blood donors with latex RAST gave a prevalence of 3.5% (Senna et al. 1999). The specificities of the latex RAST and AlaSTAT methods vary from under 70% to 97%, and due to possible false positive results these NRL allergy frequencies should be interpreted with caution (see Table 3).

Health care workers are a well-known risk group for NRL allergy. In hospital employees the frequency of NRL allergy has varied from 2.9% to 17% (Turjanmaa 1987, Arellano et al. 1992, Lagier et al. 1992, Yassin et al. 1994, Liss et al. 1997). One study of hospital personnel reported that the frequency of occupational asthma to NRL was as high as 2.5% (Vandenplas et al. 1995). A recent large prospective study of health care workers showed that about 1% of the workers became sensitized to NRL within a year (Sussman et al. 1998). In Finland, incidence rates of occupational contact urticaria

caused by NRL have been 3.9 in physicians and 2.2 in nurses per 10,000 employed worker years (Jolanki et al. 1999). In other occupations, where workers are regularly exposed to NRL gloves, the frequency of NRL allergy has been found to be 5% in greenhouse workers (Carrillo et al. 1995), 8% in housekeeping personnel (Sussman et al. 1995), 1%-10% in hairdressers (van der Walle and Brunsveld 1995, Kanerva and Leino 1999) and 11% in glove factory workers (Tarlo et al. 1990).

Table 4. Frequency of NRL allergy in adult populations.

Population	Screening test	Subjects (n)	Frequency	Author(s)
Unselected surgical patients	SPT	804	0.12%	Turjanmaa et al. 1995
Subjects with no risk factor	SPT	272	0.4%	Moneret-Vautrin et al. 1993
Patients examined for atopy	SPT	4708	0.85%	Turjanmaa et al. 1995
Allergy and asthma patients	SPT	224	4.5%	Hadjiliadis et al. 1995
Atopic subjects	SPT	180	9.4%	Moneret-Vautrin et al. 1993
Hospital employees	SPT	512	2.9%	Turjanmaa 1987
Hospital employees	SPT	224	17%	Yassin et al. 1994
Hospital employees	SPT	1351	12.1%	Liss et al. 1997

In addition to frequent exposure to NRL products, atopy is a significant risk factor for NRL allergy in adult patients. NRL-allergic health care workers are atopics from 2.2 to 4.2 times more often than their co-workers with no NRL allergy (Turjanmaa et al. 1996, Liss et al. 1997). In NRL-allergic adults, hand eczema is associated with NRL allergy, and the prevalence of this disorder has been as high as 82% (Turjanmaa et al. 1996).

A second important risk group for NRL allergy are children with spina bifida and other children requiring multiple surgical operations. These children are repeatedly exposed to various medical devices containing NRL, such as medical gloves and catheters. The frequency of NRL allergy in these children ranges from 23% to 65% (Table 5, Yassin et al. 1992, Kelly et al. 1993, De Swert et al. 1997, Mazon et al. 1997). These NRL allergy frequency rates are mainly based either on SPT screening or on IgE measurements by serological methods. In the studies based on questionnaires the frequency has been lower, varying from 0% to 22% (Meeropol et al. 1993, Pearson et al. 1994). The explanation for this seems to be the fact that not all children with NRL allergy exhibit clinical symptoms (Yassin et al. 1992, Nieto et al. 1996). For some unknown reason, the NRL allergy frequencies in children with spina bifida have been lower in Europe than in the USA, and a very low prevalence rate, 4.3%, has been reported from Venezuela (Capriles-Hulet et al. 1995).

During recent years, several studies have examined the risk factors for NRL allergy in children with spina bifida (Michael et al. 1996, Nieto et al. 1996, De Swert et al. 1997,

Mazon et al. 1997). These studies have shown that the number of surgical operations is the main risk factor in these children. Bode et al. (1996) reported that children who had been operated at least once within the first 6 months of life had a significantly increased risk of sensitization to NRL. Michael et al. (1996) showed that more than five operations significantly increased the risk. Kelly et al. (1994) recognized that nine or more surgical procedures increased the risk of an anaphylactic reaction. Atopy has also been shown to be a major risk factor for NRL allergy in children with spina bifida and it may act either as an individual or synergistic risk factor (Michael et al. 1996, Nieto et al. 1996, Bode et al. 1996, De Swert et al. 1997). Liebke et al. (1996) reported that atopic dermatitis could be a risk factor for sensitization to NRL in children.

Table 5. Frequency of NRL allergy in children with spina bifida.

Population	Test	Subjects	Frequency	Authors
Spina bifida	SPT	76	65%	Yassin et al. 1992
Spina bifida	SPT	83	51%	Kelly et al. 1993
Spina bifida	Glove challenge	74	23%	De Swert et al. 1997
Spina bifida	SPT and CAP RAST	110	29%	Mazón et al. 1997

There are only a few NRL allergy frequency studies on children who have no history of multiple operations. Two case reports of NRL allergy in atopic children have been published earlier (Table 6, Axelsson et al. 1988, Sorva et al. 1995). Using NRL glove extract for SPT, Shield and Blaiss (1992) examined 80 consecutive children referred for inhalant allergy testing and found three (4%) children with positive SPT (Table 6). However, only one of these children had no history of surgical operations. Akasawa et al. (1993) measured IgE antibodies to NRL in sera from 304 children with atopic dermatitis, asthma and/or food allergy. Twelve (4%) children showed IgE antibodies and five (1.6%) sera were confirmed to be clearly positive (score >2) with latex CAP RAST. Liebke et al. (1996) screened 609 atopic and non-atopic children with latex CAP RAST and found as many as 61 (10%) children with a positive test. Only 12 (2%) of these children were confirmed to have NRL allergy with the glove challenge test. Insulin vial stoppers contain NRL and thus the frequency of NRL allergy was studied in 112 children with type I diabetes (Danne et al. 1997). IgE antibodies to NRL were found in seven (6%) children, all of whom were atopics.

Table 6. Reports of NRL allergy in children without a history of multiple operations.

Population	Test	Subjects	Frequency	Authors
Atopic children	SPT, RAST	3	-	Axelsson et al. 1988
Children referred for evaluation for inhalant allergy	SPT	80	4%	Shield and Blaiss 1992
Atopic children	RAST	304	4%	Akasawa et al. 1993
Atopic children	SPT, CAP RAST	11	-	Sorva et al. 1995
Atopic and non-atopic children	Glove challenge	609	2%	Liebke et al. 1996
Children with type I diabetes	CAP RAST	112	6%	Danne et al. 1997

5. NRL allergens

5.1. Source of NRL and its use in rubber manufacture

NRL is formed in the cytoplasm of laticiferous cells which occur beneath the bark of the rubber tree, *Hevea brasiliensis* (Hamann 1993, Subramaniam 1995). Fresh NRL consists of rubber hydrocarbon particles in an aqueous solution commonly called as the "serum phase". There are also numerous non-rubber particles called lutoids. Rubber hydrocarbon (cis-1,4-polyisoprene) is surrounded by proteins and lipids, and these together form the rubber particles. Non-rubber substances include proteins (1-1.8%), carbohydrates, lipids and inorganic constituents. NRL is obtained from the rubber trees by tapping in which the bark is cut and then liquid latex flows from the cut along a spout to a cup. Soon after tapping and collecting the latex, a stabilizer (e.g., ammonia, and/or tetramethylthiuram disulfide) is added to prevent bacterial growth. Thereafter the NRL is ready for processing and manufacture. The NRL is processed either to a solid, dry rubber or a liquid latex concentrate. During manufacture, different chemicals, such as accelerators, vulcanization agents, activators, stabilizers and antioxidants, are added to both dry rubber and latex concentrate. Products such as conveyor belts, car tires and rubber hoses are manufactured from dry rubber. Dipped products, like gloves, condoms, catheters and balloons, are made from latex concentrate.

5.2. Allergens in NRL

More than 50 proteins in NRL have been demonstrated to bind to IgE antibodies from NRL-allergic patient sera (Alenius et al. 1994c). An allergen is termed a major allergen if it binds to IgE from the sera of over 50% of the respective patient group. At present, several important allergens have been characterized in NRL, but knowledge about the allergens and their concentration in manufactured NRL products such as gloves is scanty. Nine NRL allergens, eight of which have a nomenclature designation on the official Nomenclature List, are described in Table 7.

Table 7. Names, structure and clinical importance of NRL allergens.

Systematic / conventional name of the allergen	MW (kD)	Function as NRL allergen and structural homology	Sensitized patients	Authors
Hev b 1/ Rubber elongation factor	14.6	Major allergen in multioperated children, in HCW? Tightly bound to rubber particles	67% SB (ELISA), 81% SB (EAST) 10% A+C(ELISA), 52% HCW (EAST)	Alenius et al. 1996a, Chen et al. 1997b
Hev b 2/ ß-1,3-glucanase	36	Defence-related protein Homology to several plant ß-1,3-glucosidases	21% A+C (IB)	Alenius et al. 1995a
Hev b 3/ 23 kD Rubber particle protein	23	Major allergen in children with spina bifida Homology to Hev b 1	80% SB (IB), 76% SB (ELISA) 1/5 HCW (ELISA)	Alenius et al. 1993, Lu et al. 1995
Hev b 4/ Microhelix protein complex	50-57	Significance as NRL allergen unknown		Sunderasan et al. 1995
Hev b 5/ Acidic C-serum protein	16	Major allergen in children with spina bifida and HCW, homology to kiwi fruit protein	52% HCW (IB) 92% HCW and 56% SB (RAST)	Akasawa et al. 1996, Slater et al. 1996
Hev b 6.01/ Prohevein	20	Major NRL allergen	69% A+C, 84% HCW, 48% SB (ELISA)	Alenius et al. 1996c, Banerjee et al. 1997
Hev b 6.02/ Hevein, prohevein N-domain	4.7	Major NRL allergen. Homology to several chitin-binding proteins in plants	56% A+C, 88% HCW, 56% SB (ELISA) 75% HCW, 27% SB (EAST)	Alenius et al. 1996c, Banerjee et al. 1997, Chen et al. 1997a
Hev b 6.03/ Prohevein C-domain	14	Homology to wound-inducible proteins (WIN 1 in potato)	21% A+C, 40% HCW, 28% SB (ELISA)	Alenius et al. 1996c, Banerjee et al. 1997
Hev b 7/ Patatin-like protein	46	Homology to patatin storage proteins in potato and tomato	23% HCW (IB)	Beezhold et al. 1994, 1996
Hev b 8/ Profilin	14	Significance as an allergen unknown	11% HCW (IB)	Vallier et al. 1995
Hevamine	30	Minor allergen	3% A+C (IB)	Alenius et al. 1995a

SB = children with spina bifida, A = adults, C = children, HCW = health care workers
IB = immunoblotting, EAST = enzyme allergosorbent test

Hev b 1, rubber elongation factor (REF). This was the first NRL allergen identified (Czuppon et al. 1993). It is a highly hydrophobic protein and tightly bound to rubber particles. Hev b 1 has been shown to be a major IgE-binding allergen in children with spina bifida and other children requiring multiple operations (Alenius et al. 1996a, Yeang et al. 1996). Its importance in NRL-allergic adults still remains open. Alenius et al. (1996a) found IgE antibodies to Hev b 1 in only a small number of adult patients, whereas Chen et al. (1997b) detected antibodies in 52% of 105 NRL-allergic health care workers.

Hev b 2, β-1,3-glucanase. This allergen belongs to the defence-related proteins which are responsible for pathogen resistance in several plants (Yagami et al. 1998). Hev b 2 is structurally homologous to several plant β-1,3-glucanases and may thus play a role as a cross-reactive NRL allergen (Alenius et al. 1995a, Posch et al. 1998, Breiteneder and Scheiner 1998).

Hev b 3, 23 kD rubber particle protein. The biological function of this allergen, also described in the previous immunoblot studies as the 27 kD NRL allergen (Alenius et al. 1993), is still unknown. It has structural homology to Hev b 1 and is also tightly bound to rubber particles. Hev b 3 has been shown to be a major NRL allergen in children with spina bifida, but it seems to have only a minor role in NRL-allergic adults (Alenius et al. 1993, Lu 1995).

Hev b 4, microhelix protein complex. This is an acidic protein and a component of microhelix protein complex occurring in B-serum, i.e., enriched in lutoid particles (Sunderasan et al. 1995). Its significance as an NRL allergen has not been determined.

Hev b 5, acidic C-serum protein. Akasawa et al. (1996) and Slater et al. (1996) identified this 16 kD acidic protein and found it as a major allergen both in the health care workers and children with spina bifida. Structural homology (47% sequence identity) exists between Hev b 5 and an acidic kiwi fruit allergen (SwissProt P43393).

Hev b 6.01, prohevein; Hev b 6.02, hevein; Hev b 6.03, prohevein C-domain. Prohevein is a two-domain 20 kD protein that is processed into the N-terminal 4.7 kD domain called hevein and into the C-terminal 14 kD domain. Hevein is a hydrophilic, chitin-binding protein which can inhibit fungal growth. It is also involved in the coagulation of NRL. Hevein has been shown to occur in and eluting from the NRL gloves (Alenius et al. 1996c). Prohevein and hevein are major allergens in NRL-allergic patients, whereas the C-domain seems to be a minor allergen (Alenius et al. 1996c, Banerjee et al. 1997, Chen et al. 1997a, Posch et al. 1998, Breiteneder and Scheiner 1998). Hevein has structural homology to lectins such as wheat germ agglutinin and plant endochitinases, and it seems to play a significant role in "latex-fruit" cross-reactivity (Mikkola et al. 1998, Diaz-Perales et al. 1998, Chen et al. 1998). The C-terminal domain of prohevein is homologous to wound-inducible proteins in potato (win 1) (Breiteneder and Scheiner 1998).

Hev b 7, patatin-like protein. Hev b 7 seems not to be a major NRL allergen because IgE antibodies have been found only in a quarter of NRL-allergic adults (Beezhold et al. 1994). Hev b 7 may be important as a cross-reactive allergen because it has sequence homology to the storage proteins, patatins, in potato and tomato (Beezhold et al. 1994, 1996).

Hev b 8, profilin. Profilins are allergens in many plant species such as grass, weed and tree pollens, and they occur also in many fruits and vegetables (Vallier et al 1995, Breitener and Scheiner 1998). Profilin has also been found in NRL. Its role as an NRL allergen is, however, questionable because only a few of the NRL-allergic patients have shown IgE antibodies to profilin (Vallier et al. 1995). The profilins in NRL and ragweed have structural homology, and profilin may also be involved in cross-reactivity between NRL and banana (Vallier et al. 1995).

Hevamine. This is a protein with lysozyme and chitinase activity which is homologous to defence-related proteins in other plants. Hevamine has been isolated and purified from NRL. Hevamine is not regarded as an important allergen because IgE antibodies have been found only in a minority of NRL-allergic patients (Alenius et al. 1995a, Posch et al. 1998).

5.3.   Allergens in NRL products

It is known that both the protein and allergen content can vary considerably in NRL gloves (Alenius et al. 1994b, Yunginger et al. 1994). Twenty to 100-fold differences have been demonstrated in protein concentrations of various NRL glove brands (Turjanmaa et al. 1995). In addition, RAST inhibition studies have shown more than 3,000-fold differences in the NRL allergen content of different glove brands (Yunginger et al. 1994). It is evident from several studies that the total protein content does not necessarily correlate with the NRL allergen content of the glove. Some NRL glove brands have been described with a rather high total protein content but a low NRL concentration and *vice versa* (Alenius et al. 1994b, Lundberg et al. 1995, Baur et al. 1997). Other proteins may also be added to the NRL during glove manufacture, a fact that is not widely recognized (Subramaniam 1995), and added protein increases the total protein content of this particular glove brand.

The allergen content of NRL gloves has been evaluated and measured by different methods. Turjanmaa et al. (1988) compared the allergenicity of 19 surgical and household gloves by using their extracts in skin prick testing in NRL-allergic adults. The results showed great variation in the allergenicity of these glove brands. The same method was used to examine different condom brands and baby pacifiers (Turjanmaa and Reunala 1989b, Turjanmaa et al. 1993). Yunginger et al. (1994) studied 71 lots of NRL glove brands and other rubber products using RAST inhibition and found that powdered gloves and balloons had higher levels of NRL allergens than other medical (e.g., anaesthesia breathing bags, intravenous tubes) and non-medical (e.g., baby pacifier, baby bottle teats, condoms) products. SPT, RAST inhibition and ELISA inhibition methods were used to study the allergenicity in 20 brands of medical gloves marketed in Finland in 1994-95 (Palosuo et al. 1998). The results showed that NRL gloves could be divided into three groups, i.e., to those containing low, moderate or high levels of NRL allergens.

At present, there is some knowledge about the presence of the specific NRL allergens in NRL gloves. Hev b 1 (Czuppon et al. 1993), Hev b 3 (Lu et al. 1995), Hev b 5 (Akasawa et al. 1996) and Hev b 6.02 (Alenius et al. 1996c) allergens have been demonstrated in NRL glove eluates. Hev b 6.02 seems to be a particularly stable NRL allergen during manufacture and due to this property it is an important allergen also in the end products such as gloves (Alenius et al. 1996c). Also Hev b 5 seems to be a very heat-stable molecule (Akasawa et al. 1996). Hev b 1 has been shown to form high

molecular weight aggregates during glove manufacture, and it was speculated that the allergenicity of Hev b 1 could be enhanced due to this property (Chen et al. 1997b). Hev b 3 is present in NRL glove extracts and glove powders, but during preservation or manufacturing this protein may be broken up to smaller peptides though IgE-binding epitopes remain still preserved (Lu et al. 1995, Yeang et al. 1996). It is of interest that new allergenic epitopes may also be formed during glove manufacture. Supporting this, Mäkinen-Kiljunen et al. (1992b) demonstrated by immunoblotting that one IgE binding allergen existed in the glove eluate but not in the crude NRL.

6. Cross-reactivity of NRL and food allergens

Patients with NRL allergy are mostly atopics and they frequently show positive SPTs to various foods of plant origin. Blanco et al. (1994) reported immediate food hypersensitivities in 52% of 25 NRL-allergic adult patients. More than half of these patients had experienced serious systemic reactions from the foods. The most common allergies verified by clinical history and SPTs were to avocado (36%), chestnut (36%), banana (28%), kiwi fruit (20%) and papaya (12%). Blanco et al. (1994) suggested the term "latex-fruit syndrome" for the simultaneous occurrence of allergic reactions to NRL and various fruits. Beezhold et al. (1996) examined the frequency of food allergies in 47 NRL-allergic health care workers and found that 70% of them had a positive SPT to at least one food. The frequencies were 53% to avocado, 40% to potato, 38% to banana, 28% to tomato and to chestnut and 17% to kiwi fruit. They found that 11 of their 47 patients had experienced anaphylactic reactions from the respective fruits whereas the majority, from 50% to 92% of the patients, were clinically asymptomatic. At present, it seems that allergic reactions to avocado, banana, kiwi fruit and chestnut are the most frequently reported food sensitivities in the adult NRL-allergic patients, but the fruit allergies in children have not been well characterized (De Swert et al. 1997). In addition, clinical allergies and/or positive SPT reactions to potato, tomato, papaya, pineapple, mango, peach, passion fruit, apple, melon and celery have also been associated with NRL allergy (Beezhold et al. 1996, Warshaw 1998).

RAST inhibition studies have confirmed the occurrence of cross-reactivity between the allergens in NRL, avocado, chestnut and banana and various other fruits (M'Raihi et al. 1991, Rodriguez et al. 1993, Blanco et al. 1994, Mäkinen-Kiljunen 1994, Ahlroth et al. 1995, Brehler et al. 1997). Mäkinen-Kiljunen (1994) demonstrated for the first time the existence of structurally similar antigens and allergens in NRL and banana by immunoelectrophoretic methods. Later, several cross-reacting fruit allergens have been identified by immunoblot inhibition (Ahlroth et al. 1995, Alenius et al. 1996b, Delbourg et al. 1996, Möller et al. 1998). During recent years, several major NRL allergens have been characterized and this has stimulated the cross-reactivity studies which have been performed also with purified allergens and ELISA inhibition assays. At present there is much evidence that at least part of this cross-reactivity between NRL and various fruits is based on structural homology between hevein and plant class I endochitinases (Mikkola et al. 1998, Diaz-Perales et al. 1998, Chen et al. 1998, Blanco et al. 1999, Posch et al. 1999). In addition, a high sequence homology between 46 kD NRL allergen, known at present as Hev b 7, and patatin of potato has been demonstrated by Beezhold et al. (1996).

C. AIMS OF THE PRESENT STUDY

This study is based on a series of 42 NRL-allergic children, 30 of which non-operated and 12 multioperated, diagnosed at the Department of Dermatology, Tampere University Hospital, in 1988-1995, and the specific aims were as follows:

1. To determine the frequency of NRL allergy in children admitted for inhalant or food allergy testing including NRL allergen to routine SPT series.

2. To compare clinical findings, SPT and latex RAST results between non-operated and multioperated NRL-allergic children.

3. To examine the frequency of IgE antibodies to a selection of purified NRL allergens in NRL-allergic children.

4. To study the clinical outcome, and to examine whether SPT reactivity and IgE antibody responses are decreased in NRL-allergic children when they avoid NRL products in everyday life and in hospital environment during the follow-up.
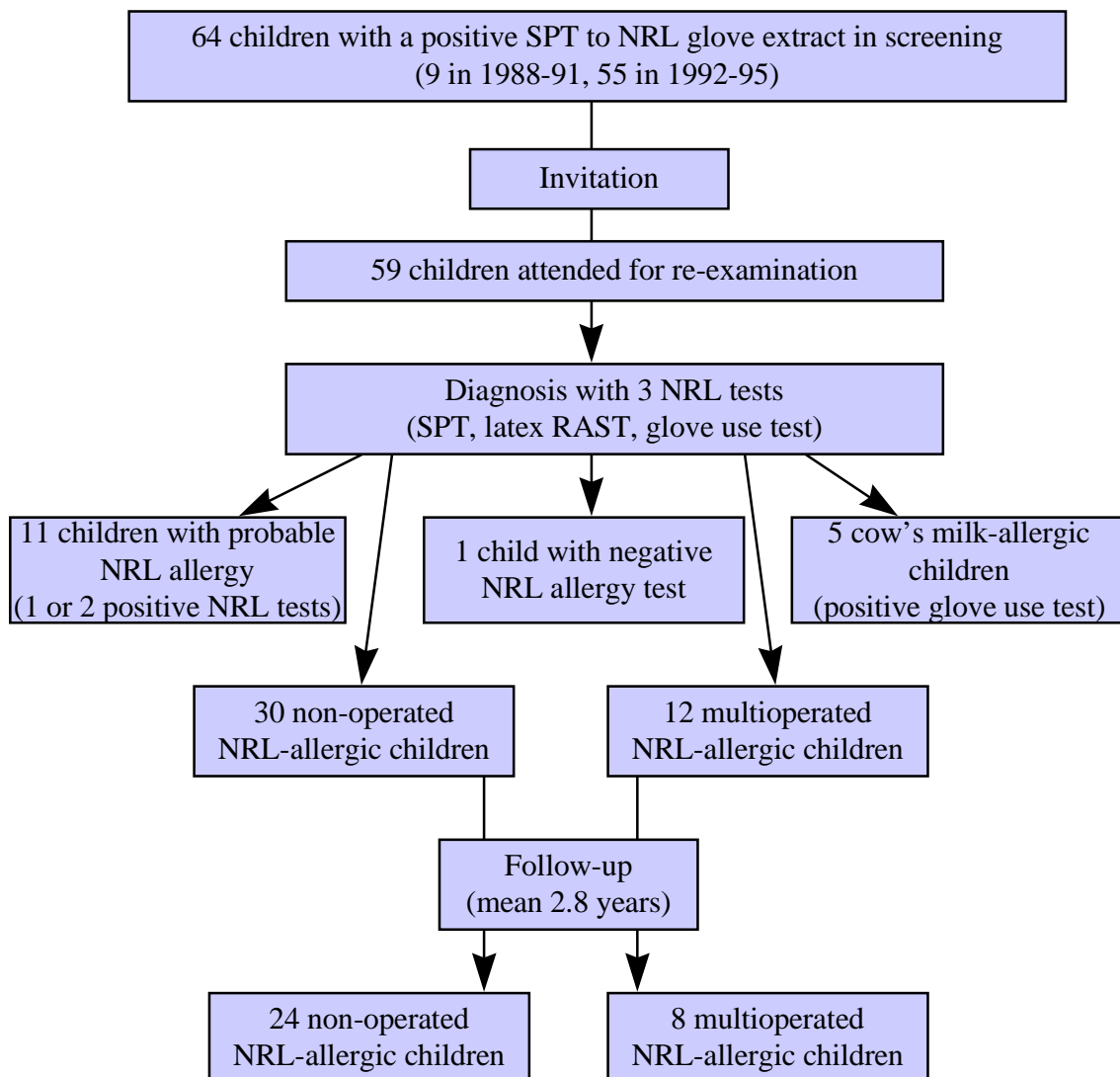
D. MATERIALS AND METHODS

1. Patients

1.1.   Selection of NRL-allergic children (I, IV)

In order to screen for NRL allergy among children admitted to the allergy laboratory at the Department of Dermatology, Tampere University Hospital, NRL glove extract was included in the inhalant SPT series in 1988 and in the basic food allergen SPT series in 1992. These series are routinely used to test children suspected of having inhalant or food allergies and various atopic disorders. SPT with NRL glove extract was positive in 9 children in 1988-91, and in 55 children in 1992-95. Twelve of the children were admitted because of symptoms of NRL allergy, and the remaining 52 children were found due to SPT screening (Flow-chart 1).

Flow-chart 1. Number of NRL-allergic children diagnozed and followed in the study.

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┌─────────────────────────────────────────────────────────┐
│ 64 children with a positive SPT to NRL glove extract in  │
│ screening                                                │
│ (9 in 1988-91, 55 in 1992-95)                            │
└─────────────────────────────────────────────────────────┘
                          │
                  ┌───────────────┐
                  │  Invitation   │
                  └───────────────┘
                          │
          ┌───────────────────────────────────┐
          │ 59 children attended for re-examination │
          └───────────────────────────────────┘
                          │
              ┌─────────────────────────┐
              │ Diagnosis with 3 NRL tests │
              │ (SPT, latex RAST, glove use test) │
              └─────────────────────────┘
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| 11 children with probable NRL allergy (1 or 2 positive NRL tests) | 1 child with negative NRL allergy test | 5 cow's milk-allergic children (positive glove use test) |

| 30 non-operated NRL-allergic children | 12 multioperated NRL-allergic children |

Follow-up
(mean 2.8 years)

| 24 non-operated NRL-allergic children | 8 multioperated NRL-allergic children |

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Fifty-nine (92%) of the 64 children with a positive SPT to NRL glove extract were re-examined in 1995-96 (I). The personal history of atopy (past or present atopic dermatitis and/or allergic rhinitis and/or asthma), symptoms of NRL allergy and their causes were registered using a structured questionnaire. The number of surgical operations and underlying disorders were recorded, and all unexpected intraoperative events were verified from the hospital records. The final NRL allergy diagnosis was based on three criteria: (1) a positive SPT to at least one of the two NRL allergens used in the testing, (2) a positive latex RAST and (3) a positive NRL glove use test (see Methods). On the basis of these strict criteria, 42 (71%) of the 59 children were diagnosed as having NRL allergy. For a more detailed analysis, the NRL-allergic children were further divided into two groups; the 30 children without and the 12 children with a history of multiple operations. The third group consisted of 11 children who had only one or two positive NRL allergy tests, and this group was considered to have probable NRL allergy. In addition, five children, all allergic to cow´s milk, were interpreted as having false positive results in the glove use test due to casein in the gloves (IV). One child upon re-examination had negative results on all the NRL tests and was excluded from further studies.

1.2. Follow-up of NRL-allergic children (V)

A prospective follow-up study was performed to examine the outcome of the 42 NRL-allergic children. At diagnosis, the parents of these children had been carefully advised on how to avoid NRL products in everyday life and in hospital. A warning of NRL allergy was attached to the hospital records, and all surgical procedures were advised to be performed in a NRL-free environment. The parents and children had been also advised to contact our department if any problems arose with protection or symptoms of NRL allergy. Seven NRL-allergic children visited our department regularly because of atopic dermatitis and/or food allergy.

A total of 32 NRL-allergic children, 24 non-operated and 8 multioperated children, could be evaluated at the end of the follow-up in 1998. The mean follow-up time was for the non-operated children 2.8 years (range 2.3-3.1 years) and for the multioperated children 2.9 years (range 2.7-3.1 years). When re-examined at the end of follow-up, all children and/or their parents were personally interviewed using a structured questionnaire. Exposure to and symptoms from NRL products were recorded, and the number of surgical operations and their outcome were also checked from the hospital records. In addition, SPTs were performed and sera taken for specific IgE antibody measurements.

2. NRL allergy tests

2.1. Skin prick test (I, III, IV, V)

The screening for latex allergy was performed with a latex glove extract (1:5 w/v, Triflex[R], Baxter, Valencia, CA, USA). A fresh extract was prepared every month as described previously (Turjanmaa 1988, 1995), using the same lot of gloves (lot 06 92L12DPGN) since 1992. At diagnosis, a prick test was performed also with a commercial latex allergen (Stallergènes SA, Fresnes, France) which has been standardized by *in vivo* and *in vitro* methods by Turjanmaa et al. (1997). In addition, prick tests were performed with seven common inhalant allergens (birch, timothy and

mugwort pollens; cat and dog danders; *Dermatophagoides pteronyssinus* and *D. farinae*; Allergologisk Laboratorium a/s, ALK, Hørsholm, Denmark). Banana, kiwi fruit, avocado and potato prick tests were performed with freshly peeled fruit/vegetable by the prick-prick method (Dreborg and Foucard 1983).

Five children in Study IV were skin prick-tested with the two NRL allergens (Triflex glove extract, Stallergènes SPT reagent) and also with cow's milk allergen (ALK).

At follow-up examination (V), SPTs were performed with the same two NRL allergens (Triflex glove extract, Stallergènes SPT reagent) as at diagnosis. Also banana, kiwi fruit, avocado and raw potato SPTs were performed as mentioned above. SPT with purified Sol t 1 (30 and 200 µg/ml) was done to 27 NRL-allergic children (III).

All prick tests were performed on the forearm using a commercial one-peak lancet (ALK) as described by Turjanmaa (1988). Histamine dihydrochloride (10 mg/ml, ALK) served as a positive control and physiological saline as a negative control. The mean diameter was measured at 15 min, and a wheal half the size of that of histamine and at least 3 mm was regarded as positive.

2.2.　Latex RAST (I, IV, V)

A commercial latex RAST and also total IgE assays were performed with the CAP method (Pharmacia, Uppsala, Sweden). The measurements were performed in the routine clinical laboratory and latex RAST levels > 0.4 kU/L were considered positive (I, V). In Study IV, serum IgE antibodies to cow's milk and casein were also measured by CAP RAST.

2.3.　Glove use test (I)

The use test in Study I was started with a finger piece of NRL glove (Triflex) applied on a wetted finger for 15 min. The test was regarded as positive if two or more wheals were seen on the contact area. If the test was negative, the challenge was continued with a piece of glove around the wrist for 15 min. If this remained negative, the challenge was continued for one to five hours a day up to one week. A piece of vinyl glove was used as a negative control in every use test.

3. Purification of NRL allergens

3.1.　Hev b 6.01, Hev b 6.02 and Hev b 1 (II, V)

Prohevein (Hev b 6.01) was purified from the bottom (lutoid) fraction of fresh, ultracentrifuged NRL as described previously by Alenius et al. (1995a). Briefly, NRL was centrifuged at 43,000 x *g* for 1 h, the supernatant was collected, dried in a vacuum centrifuge, redissolved and filtered (0.45 µm Millex-HV Millipore, Molsheim, France). The sample was then subjected to high-performance liquid chromatography (HPLC) gel filtration on a Superdex 75 HR 10/30 column (Pharmacia). The fraction containing the 20 kD NRL protein was collected and further purified by reversed-phase HPLC using a linear gradient of acetonitrile (20-35% in 40 min) in 0.1% trifluoroacetic acid. The fractions containing prohevein were then dried in a vacuum centrifuge and stored at -20ºC before use in ELISA.

Hevein (Hev b 6.02) was purified from the bottom fraction of fresh ultracentrifuged NRL as described by Alenius et al. (1996c). Briefly, the sample was subjected to reversed-phase HPLC on a ProRP 5/2 column (Pharmacia) using a linear gradient of acetonitrile (constant 3% concentration for 20 min and then 3-100% gradient in 10 min) in 0.1% trifluoroacetic acid. The most hydrophilic peptides were collected, dried, redissolved and then subjected to gel filtration on a Superdex Peptide HR 10/30 column (Pharmacia). The fractions containing hevein were collected, pooled and then subjected to final purification in reversed-phase HPLC on a PepRP 5/5 (Pharmacia) column. The fractions containing hevein were then collected, dried and stored at - 20ºC before use in ELISA.

REF (Hev b 1) was purified from rubber particle fraction as described by Alenius et al. (1996a). Briefly, NRL was ultracentrifuged at 43,000 x g for 1 h, the rubber particle fraction washed with ammonia, centrifuged as above in the presence of sodium dodecyl sulfate (SDS), and the proteins in the REF-enriched supernatant were separated by SDS-polyacrylamide gel electrophoresis (Mini Protean II Cell; Bio-Rad) and stained with Coomassie brilliant blue. The 14 kD protein band was excised and subjected to electroelution (Electro-Eluter, Bio-Rad) against 50 mM ammonium bicarbonate containing 0.1% SDS using 12.5 kD cut-off membrane caps. The eluted fractions containing REF were pooled, dried and stored at -20ºC before use in ELISA.

3.2. Hev b 5 and Hev b 7 (III, UR)

Hev b 5 was purified from stored deep-frozen NRL (Alenius et al. 1995a) by two consecutive runs in reversed-phase HPLC using a linear gradient of acetonitrile in 0.1% trifluoroacetic acid (first 0-100% in 20 min, then 0-60% in 60 min) in a 3 ml Resource RPC column (Pharmacia). The identity and purity of the eluted Hev b 5 were verified by amino acid sequencing and mass spectrometry as described previously (Seppälä et al. 1999).

Hev b 7, the patatin–like NRL allergen, was purified from NRL C-serum using gel filtration chromatography (16 x 60 mm Superdex 75 Fast Flow column, Pharmacia), after which the chosen fractions were pooled and run in hydrophobic interaction chromatography in a 1 ml RESOURCE[TM] PHE column (Pharmacia) with a decreasing linear gradient. The chosen fractions were pooled, desalted in a 1 x 10 cm BioGel P-6DG column (Bio-Rad Laboratories, Hercules, CA, USA) for anion exchange chromatomatography which was performed on a Mono Q HR5/5 column (Pharmacia). The purified protein was quantified by reversed-phase HPLC on a 0.21 x 10 cm TSK TMS 250 column (TosoHaas Corporation, Tokyo, Japan) with a linear gradient of acetonitrile (3-100%, in 60 min) in 0.075% trifluoroacetic acid. The protein peaks were integrated and the protein amount calculated by comparison of the peak areas to areas obtained with known amounts of bovine serum albumin and ovalbumin. The buffer exchanges for ELISA experiments (to 50 mM sodium carbonate buffer, pH 9.6), for skin prick testing (to 10 mM phosphate buffer, 150 mM NaCl, pH 7.5 [phosphate-buffered saline, PBS]) and for immunoblot studies (to 10 mM Tris-HCl, 150 mM NaCl pH 7.5) were performed by gel filtration in a 1 x 10 cm BioGel P-6DG column (Bio–Rad). For peptide mass finger printing or internal sequencing the protein to be identified was alkylated and digested with endoproteinase Lys-C, and the protein digest was then desalted using a microtip reversed-phase HPLC column. The mass-mapping analysis of the protein digest was performed by matrix-assisted laser desorption/ionisation time-of-

flight mass spectrometry (MALDI-TOF MS) using a Biflex II instrument (Bruker Franzen Analytik, Bremen, Germany). Peptide sequencing was performed using an Applied Biosystems 494 A Procise[TM] sequencer (PerSeptive Biosystems, Foster City, CA, USA).

Patatin of potato tuber (Sol t 1) was purified as described by Seppälä et al. (1999).

4. IgE antibody measurements

4.1. Serum samples (II, III, V, UR)

To examine the IgE antibody pattern to specific NRL allergens (Hev b 1, 5, 6.01, 6.02), sera were sampled at re-examination from the 30 non-operated and 12 multioperated children (II, UR), and at the end of the follow-up (V) from 24 and 8 of these children, respectively.

Control sera were obtained from 19 atopic children (mean age 6.9 years, range 1.5 to 15.5 years), who had positive SPT reactions to inhalant or food allergens but no history of NRL allergy and negative SPT to NRL. Eleven of these children had positive SPT to inhalant allergens (grass pollens, animal dander or house dust mite), 5 to banana and 3 to wheat flour (II). Seventeen of these 19 sera were used as control sera in Study V.

Sera sampled from the 35 NRL-allergic children (III) at the end of the follow-up were examined for IgE antibodies to the patatin-like NRL allergen Hev b 7, and also to a cross-reactive allergen in potato, i.e., to patatin (Sol t 1). Three of these 35 sera were not included in Study V. Control sera were obtained from 11 atopic children (mean age 2.5 years; range 5 months to 6 years) with negative SPT to raw potato and NRL but positive SPT to various other allergens such as milk, egg and/or cereals.

4.2. Immunoblotting (II)

Fresh NRL of rubber tree (*Hevea brasiliensis*) was collected in Malaysia and used in immunoblotting (Study II) as described by Alenius et al. (1993). Briefly, the dissolved NRL was electrophoresed through a 12% SDS-polyacrylamide gel and the separated proteins were transferred to nitrocellulose membranes (Trans-Blot, Bio-Rad Laboratories, Richmond, CA, USA). These were cut into strips and incubated first with the patient and control sera (diluted 1:5), then with biotinylated antihuman IgE antibody (Vector, Burlingame, CA, USA; diluted 1:2,000) and with streptavidin-conjugated alkaline phosphatase (Bio-Rad; diluted 1:5,000). Finally, a color development solution was added.

4.3. ELISA assays (II, III, V, UR)

IgE ELISA against purified allergens was performed as described by Alenius et al. (1996a,c). Briefly, prohevein (2 µg/ml in 50 mM sodium carbonate buffer, pH 9.6), hevein (1.5 µg/ml or 2.0 µg/ml), REF (1.5 µg/ml), Hev b 5 (1 µg/ml), Hev b 7 and Sol t 1 (both 2 µg/ml) were applied to 96-well polystyrene microtiter plates (100 µl/well; Nunc, Roskilde, Denmark) and incubated for 3 h at room temperature and overnight at 4ºC. The wells were then emptied and postcoated for 1 h with 100 µl of 1% human serum albumin in 50 mM carbonate buffer, pH 9.6. After washing three times with PBS

containing 0.05% Tween 20, 100 µl of patient or control serum (diluted 1:10) was added to the wells and incubated for 2 h at room temperature. After washing three times, biotinylated antihuman IgE (Vector, diluted 1:1,000) was added and incubated for 1 h. The wells were washed and streptavidin-conjugated alkaline phosphatase (Bio-Rad; diluted 1:3,000) was incubated for 1 h. After washing, the substrate development solution (Sigma Chemical Co., St. Louis, MO, USA) was added and the optical density (OD) was read at 405 nm using an automated ELISA reader (Titertek Multiskan, Eflab, Espoo, Finland). The mean OD + 3 SD of the 19 (II, UR), 17 (V) and 11 (III) controls was chosen as the cut-off limit for positivity.

5. Casein in NRL gloves (IV)

5.1. NRL gloves

Thirty brands of NRL gloves widely marketed in 1994 (Palosuo et al. 1998) were examined for casein allergens. Twenty-eight of these were surgical and examination gloves and 2 household gloves. The glove eluates were made as previously described (Turjanmaa et al. 1995). The solutions were used for rocket radioimmuno-electrophoresis. The names of the different glove brands are given in Figure 2, Study IV.

5.2. RAST inhibition and immunoelectrophoresis

In Study IV, casein in the Triflex (lot 06 92L12DPGN) gloves was measured as allergen activity by using glove eluate as inhibitor and casein RAST discs (code f78, Pharmacia) as solid phase allergens. A serum pool from the five children was used as the source of casein IgE antibodies. Dilutions of α-casein (Sigma C-7891) ranging from 5 µg/ml to 5,000 µg/ml were used as standards. A Gammex (Ansell Ltd., Melacca, Malaysia, lot 9294 407) glove was used as a control glove because it is known for its low casein (Mäkinen-Kiljunen et al. 1993) and low NRL allergen (10 allergen units/g glove) content (Palosuo et al. 1996). Four two-fold dilutions were made from the glove eluates. Non-ammoniated NRL (protein content 9.6 mg/ml) served as a negative control. The relative casein allergen activity was calculated by the parallel line method.

Casein in the Triflex and Gammex glove eluates was also measured as precipitable protein in rocket immunoelectrophoresis (RIE) (Mäkinen-Kiljunen et al. 1992b) using rabbit IgG antibodies to casein (2.28 µl/cm^2; Pharmacia, Uppsala, Sweden). Fifteen microliters of α-casein (from 0.3 to 1,250 µg/ml) was used as the standard, and two-fold dilutions from the gloves were applied to gel wells. The casein content was calculated from the height of the rocket on the plate after protein staining with Coomassie Brilliant Blue.

Casein allergens in the 30 brands of NRL glove eluates were examined by rocket radioimmunoelectrophoresis (RRIE) (Mäkinen-Kiljunen et al. 1992b). For this, RIE was run as described above and then, instead of protein staining, the plate was consecutively incubated with a pool of 50 sera from milk-allergic patients and ^{125}I-radiolabelled anti-human IgE (Pharmacia). IgE binding to casein allergens was visualized by autoradiography. NRL and cow's milk skin test allergen were used as negative and positive controls, respectively.

The total protein content of the Triflex glove eluate was measured by Lowry's method (Alenius et al. 1994b). The NRL allergen content measured by ELISA inhibition as described by Palosuo et al. (1998) was 1,400 allergen units/ml in the glove eluate, i.e., 7,000 allergen units/g of glove.

6. Statistical analysis

The statistical methods used in the present studies were: Mann-Whitney U test (I, II, V), Fischer's exact test (II, V) and Wilcoxon's matched-pair test (V).

7. Ethics

Examination of the NRL-allergic children with SPTs, glove use tests and blood sampling (I, III, V) were approved by the Ethical Committee of Tampere University Hospital, and informed consent was obtained from the parents and/or the children.

E. RESULTS

1. Prevalence of NRL allergy in children (I)

In 1992-95, a total of 3,269 children were prick tested with NRL glove extract and 55 (1.7%) children showed a positive result (I, Table I). Fifty (91%) of these children were re-examined and 33 children were confirmed as having NRL allergy by positive SPT to NRL allergen, latex RAST and NRL glove use test. This gives a 1% prevalence of NRL allergy in the children admitted for inhalant and food allergy testing. Of the 33 NRL-allergic children, 26 were non-operated and 7 were multioperated children. In addition, 11 children were susceptible to NRL allergy because they had one to two, but not three, positive NRL tests. At re-examination, 4 of these 11 children had positive SPT to at least one of the two NRL allergens. None of these 11 children with probable NRL allergy had a history of multiple operations.

2. Comparison of findings between non-operated and multioperated NRL-allergic children (I, II, III, UR)

2.1. Clinical findings

The mean age at diagnosis was 5.7 years (range 0.6-13.7 years) in the 30 non-operated children (19 boys, 11 girls) and 8.1 years (range 0.7-13.1 years) in the 12 multioperated children (6 boys and 6 girls). Atopic disorders were very common with the frequencies of 97% and 83% in the two groups of children (I, Table II).

Five non-operated children had a history of one or two minor operations (adenotomy, hernioplasty or excision of a nevus). Seven of the multioperated children had spina bifida, two had other congenital anomalies, one child anal atresia, one hydronephrosis, and one child had been operated for club-foot. The mean number of surgical operations was 9.1 (range 5 to 15) in these children.

Eight (27%) non-operated children were admitted because of NRL allergy symptoms. Eleven children were found to have symptoms only after taking a careful history at re-examination, and the remaining 11 (37%) children had no history of symptoms. Of the 12 multioperated children, four (33%) were admitted because of NRL allergy symptoms, five were found to have had symptoms when interviewed carefully and three (25%) had been totally asymptomatic.

Symptoms from NRL products in 19 non-operated children were contact urticaria (74%), lip and facial swelling (58%), eye symptoms (21%), rhinitis (5%) and asthma (5%). The frequencies of these same symptoms in the nine multioperated children were 67%, 56%, 44%, 33% and 22%, respectively. One child with a history of multiple operations had experienced an anaphylactic reaction during surgical treatment and another child experienced contact urticaria during operation. Otherwise the operations in the remaining 10 children had gone without any symptoms.

The NRL products which caused the symptoms in the NRL-allergic children were balloons, rubber toys, elastic bandages, and surgical and household gloves (I, Table IV)

2.2. SPT to NRL and latex RAST

In the SPT the mean diameters of wheal reactions to the NRL glove extract and Stallergènes reagent were 7 mm and 6 mm in the 30 non-operated and 5 mm and 6 mm in the 12 multioperated children, respectively. In the non-operated children, the frequencies of positive SPT to pollens, animal dander and house dust mites were 90%, 73% and 33%, and in the multioperated children 25%, 25% and 8%, respectively.

Eighty-seven percent of the non-operated children had positive SPT to cross-reactive fruits. The frequencies were 63% to banana, 67% to kiwi fruit and 65% to avocado. SPT to raw potato was positive in 70% of these children. Correspondingly, 50% of the multioperated children had positive SPT to some cross-reactive fruits, and 25% of these children reacted to banana, 42% to kiwi fruit and 17% to avocado. SPT to raw potato was positive in 25% of these children.

The mean latex RAST level was 5.0 kU/L (range 0.4 to 72.2 kU/L) in the non-operated and 2.9 kU/L (range 0.7 to 74.2 kU/L) in the multioperated children, which is a non-significant difference. Similarly, the mean total IgE levels were 350 kU/L (range 27 to 3,940 kU/L) and 224 kU/L (range 24 to 2,036 kU/L) in these two groups of children, respectively.

2.3. IgE antibodies to Hev b 6.01, Hev b 6.02 and Hev b 1

In immunoblotting, 21 (70%) and 9 (30%) sera from the 30 non-operated children showed IgE antibody binding to 20 kD and 14 kD allergens, respectively. In the multioperated children the frequencies (33% and 67%) were the opposite. Serum from one child with multiple operations showed IgE antibody binding to 23/27 kD allergen (Table 8). One control serum from a child with allergy to banana showed IgE binding to the 20 kD and 14 kD NRL allergens, and another child with wheat allergy IgE binding to the 20 kD allergen.

In IgE ELISA, sera from 26 (86%) non-operated children showed elevated levels of IgE antibodies to prohevein and 19 (63%) to hevein, whereas 7 sera (58%) from the multioperated children showed IgE antibodies to both of these allergens (Table 8). The mean IgE antibody levels to prohevein and hevein did not differ significantly (p=0.22 and 0.88, respectively) between the two groups of children. Sera from 8 (27%) non-operated and 8 (67%) multioperated children showed IgE antibodies to REF (Table 8), which was a significant difference (p=0.032). The mean IgE antibody level to REF was also significantly (p=0.012) higher in the multioperated than in the non-operated group of children (Table 8).

Table 8. Frequencies of IgE antibodies to specific allergens in ELISA in 42 NRL-allergic children.

Purified NRL allergen	Non-operated children (n=30)	Multioperated children (n=12)*
Hev b 6.01	26 (86%)	7 (58%)
Hev b 6.02	19 (63%)	7 (58%)
Hev b 1	8 (27%)	8 (67%)
Hev b 5	1 (3.3%)	4 (33%)
Hev b 7	0	0

* One child showed IgE binding to the 23 kD allergen (Hev b 3) in immunoblotting but ELISA measurements could not be performed with this allergen.
The cut-off levels for positivity in ELISA are mean OD + 3 SD of the control children.

2.4.   IgE antibodies to Hev b 5 and Hev b 7

In ELISA, one (3%) serum from the 30 non-operated children and 4 (33%) from the 12 multioperated children had IgE antibodies to Hev b 5 (p=0.01). In contrast, IgE antibodies to Hev b 7 were not found in any of the 35 NRL-allergic children (Table 8).

Twenty-three (66%) sera showed IgE antibodies to Sol t 1. Twenty-two (63%) of the 35 children had positive SPT to raw potato. Twenty of these 22 children were non-operated. Eight (30%) of the 27 children tested had positive SPT to Sol t 1 and seven of these children were non-operated.

3. Children allergic to cow's milk and casein content of NRL gloves (IV)

3.1.   SPT, RAST and challenge tests

Five children with positive NRL glove use test at re-examination (I) had negative SPT results with a commercial NRL allergen and latex RAST. All five children showed a positive SPT with cow's milk allergen and positive RAST to cow's milk and casein. In open challenge with cow's milk, these children had immediate symptoms such as contact urticaria, lip and facial edema (IV, Table I).

3.2.   Casein content of gloves

In casein RAST, a distinct inhibition was observed with the Triflex glove eluate, but not with the control glove eluate or NRL control preparation. The casein content was 75 µg/mL (375 µg/g) for the Triflex and less than 1 µg/mL (5 µg/g) for the Gammex glove.

In RIE for casein antigens, a faint staining of rockets was demonstrated with the Triflex, but no staining was seen with the control glove. The casein content was 78 µg/mL (390 µg/g) for the Triflex and less than 2 µg/mL (10 µg/g) for the Gammex glove.

In RRIE for casein allergens, a distinct binding of IgE antibodies was demonstrated in 8 NRL glove brands including Triflex. Seven other glove brands showed a faint IgE binding. Fifteen glove brands were negative for casein.

The total protein (by Lowry's method) content of the Triflex glove was 1,000 µg/g.

4. Follow-up of NRL-allergic children (V)

Of the 42 NRL-allergic children at initial examination, 32 children could be followed up a mean of 2.8 years in order to examine clinical outcome, SPT reactivity and IgE antibody levels to specific NRL allergens. During the follow-up, 8 parents contacted our department due to their children's allergic reaction at home.

4.1.   Clinical findings

During the follow-up, 19 (79%) of the 24 NRL-allergic non-operated children and 3 of the 8 multioperated children had occasional contacts with balloons, rubber boots and household NRL gloves at home. Three multioperated children remained free of symptoms, whereas 10 (53%) of the non-operated children experienced symptoms such as contact urticaria and lip swelling. Two non-operated children had systemic reactions such as generalized urticaria, facial oedema and asthmatic attack from the NRL products. Two non-operated children underwent a minor operation whereas 1 to 8 operations were performed on 5 multioperated children without any symptoms.

4.2.   SPT to NRL allergens and latex RAST

In the non-operated children, the mean diameters of SPT wheal reactions to NRL glove eluate (Triflex) and to the commercial NRL allergen (Stallergènes) were 6.5 mm and 5.8 mm at diagnosis, and 6.9 mm and 6.0 mm at the end of the follow-up, respectively. In the multioperated children, the mean diameters were 6.9 mm and 6.0 mm at diagnosis, and 6.4 mm and 5.6 mm at the end of the follow-up, respectively. Therefore, the SPT reactivity to the NRL allergens did not show during the follow-up any decrease in either group of children (V, Table 2a,b).

The results of the SPT reactivity to cross-reactive fruits and potato at diagnosis and at the end of the follow-up are seen in Table 9.

Table 9. Frequencies of positive SPTs to cross-reactive fruits and potato at diagnosis and after the follow-up in 32 NRL-allergic children.

SPT	Non-operated children (n=24) At diagnosis / At follow-up	Multioperated children (n=8) At diagnosis / At follow-up
Banana	63 % / 46 %	38 % / 50 %
Kiwi fruit	63 % / 67 %	50 % / 50 %
Avocado	60 % / 54 %	25 % / 38 %
Potato	67% / 67 %	25 % / 38 %

In the non-operated children, the mean latex RAST level was 6.3 kU/L (range 0.4 to 28.4 kU/L) at diagnosis and 5.7 kU/L (range <0.4 to 20.2 kU/L) at the end of the follow-up. Correspondingly, in the multioperated children the levels were 12.0 kU/L (range 0.7 to 74.2 kU/L) and 12.9 kU/L (range <0.4 to 79.1 kU/L), respectively. The mean latex RAST levels did not show any significant change (p=0.291 and 0.575, respectively) during the follow-up in either group of children. The mean total IgE level

decreased somewhat in the multioperated children, but also in this group of children the change was not significant (p=0.161).

4.3.   IgE ELISA to NRL allergens

In ELISA, the mean IgE antibody levels to prohevein, hevein and REF were 0.317, 0.102, and 0.008 OD units, respectively, at diagnosis in the non-operated children, and after the follow-up 0.229, 0.129 and 0.001 OD units, respectively (V, Table 2a,b). In the multioperated children the mean IgE antibody levels to these NRL allergens were 0.303, 0.163 and 0.280 OD units, respectively, at diagnosis, and after the follow-up, 0.242, 0.159 and 0.208 OD units, respectively. The only significant decrease was in the mean IgE levels to REF (p=0.002) in the non-operated children.

F. DISCUSSION

1. Prevalence and diagnosis of NRL allergy in children

During 1992-95 we skin-prick tested with NRL glove extract 3,269 children admitted for inhalant or food allergy testing and found a positive test in 55 (1.7%) children (I). Fifty of these children were re-examined and using strict diagnostic criteria, i.e., a positive SPT, latex RAST and glove use test, 33 (1.0%) children were confirmed as having NRL allergy. Twenty-six of these children were non-operated and 7 had a history of multiple operations (I). In previous studies in children with spina bifida and other children requiring multiple operations, the frequency of NRL allergy has varied from 23% to 65% (Yassin et al. 1992, Kelly et al. 1993, De Swert et al. 1997), and in non-operated children from 2% to 6.3% (Shield and Blaiss 1992, Liebke et al. 1996, Bode et al. 1996). Recently, Novembre et al. (1997) performed SPT screening of 453 consecutive children referred to a university hospital clinic mostly because of atopic disorders. They found 10 (2.2%) children with positive SPT to NRL, 5 of these children had a positive latex RAST, and 3 (0.7%) children also a positive glove use test. This result agrees quite well with the results of the present study, and shows that using strict diagnostic criteria, i.e., also a glove use test, the prevalence of NRL allergy is about 1% in children who are mostly atopics. Recently, Bernardini et al. (1998) determined NRL allergy prevalence in an unselected paediatric population. When screening 1,175 children in 11 elementary schools with SPT, they found 8 (0.7%) subjects with a positive SPT to NRL, but no challenge tests were performed to confirm the diagnosis of NRL allergy.

When we screened a large population of children with SPT and then confirmed the NRL allergy diagnosis carefully, we found three times more non-operated than multioperated children with NRL allergy (I). This shows that also children without a history of multiple operations can become sensitized to NRL and that these children can be easily detected in allergy practice by performing routine skin prick testing with NRL allergen. Only one third of the NRL-allergic children were admitted because of NRL allergy symptoms and for the others the NRL allergy diagnosis was not expected. After careful interview of parents and children, symptoms of NRL allergy could be found in two-thirds of the children, but a third had a totally negative history although they showed a positive glove challenge tests (I). Thus, it is important to perform active SPT screening with NRL allergen for all children admitted for inhalant and food allergy testing, i.e., for mainly atopic children, and not only for children with spina bifida or other children belonging to the well-known risk group of NRL allergy.

The present study identified a group of children with suspected NRL allergy, i.e., 11 children who at re-examination had 1 or 2 but not all 3 NRL allergy tests positive (I). A few of these children had a positive SPT and/or latex RAST but the glove use test or clinical history was negative. This suggests that the SPT and latex RAST in these children could be "false" positive test results. Because there were no methods to confirm this and NRL allergy can have a serious outcome, as a precaution we recommended a strict avoidance of NRL products for these children. On the other hand,

five children had positive latex RAST and challenge test but negative SPT to commercial NRL reagent, suggesting that also "false" negative SPTs may occur. The SPT reagent we used had been previously standardized and the sensitivity and specificity had been found to be very good in NRL-allergic adults (Turjanmaa et al. 1997) but only moderate in children with spina bifida (De Swert et al. 1997). It seems that in children a NRL allergy diagnosis could not be based solely on a positive SPT, and especially on a weak positive SPT reaction. Supporting this, in six of the present 11 children with negative SPT at re-examination, the size of the SPT reaction in screening had been smaller than that of histamine. Moreover, the sensitivity and specificity of latex RAST or AlaSTAT are also less than 100% and false positive results may occur possibly due to cross-reacting IgE antibodies (Mäkinen-Kiljunen 1994, Beezhold et al.1996). Unexpectedly, the present study also showed that the NRL glove use test produced false positive results. The children with false positive reactions were all allergic to cow´s milk and the casein was the cause of the positive glove use test reactions (IV) (see Chapter 4). It seems evident, therefore, that diagnosis or exclusion of NRL allergy in children should not be based solely on the findings of a single NRL allergy test because SPT, latex RAST and NRL glove use test may all sometimes produce false positive results.

2. Comparison of non-operated and multioperated NRL-allergic children

The non-operated and multioperated NRL-allergic children did not differ as to sex distribution or age. Atopic disorders were common in both groups and the frequency was 97% in the non-operated and 83% in the multioperated children (I). In agreement with these high frequencies, atopy has been shown to be a clear risk factor for NRL allergy in health care workers and in children with spina bifida (Turjanmaa 1987, Moneret-Vautrin et al. 1993, Nieto et al. 1996). It has been also suggested that atopic dermatitis *per se* could be a risk factor for NRL sensitization in children (Liebke et al. 1996). In our study, the frequency of atopic dermatitis was high both in the non-operated (90%) and multioperated children (67%) (I). In atopic dermatitis the skin barrier is disrupted (Aalto-Korte 1995, Tanaka et al. 1997) which may allow allergens from NRL products to penetrate more easily into the skin and promote sensitization. It is, however, evident that the sensitization route from NRL gloves to the body could frequently be through the mucous membranes when the children with spina bifida are operated (Yeang et al. 1996). The children could also be sensitized by inhaling glove powder contaminated with NRL allergens (Turjanmaa et al. 1990), and the same could occur when the children are handling or blowing balloons.

Two-thirds of the present non-operated and multioperated children had exhibited symptoms from NRL products. The symptoms were usually mild and mainly localized to the area of contact with NRL gloves and balloons. The most common symptom in both groups was contact urticaria and, secondly, lip and facial swelling. Asthma, rhinitis and eye symptoms were uncommon. Only one child with a history of multiple operations had experienced an anaphylactic reaction during the operation (I). In the first published studies, the most frequently reported symptom in NRL-allergic children with spina bifida and other children requiring multiple operations was anaphylaxis (Slater 1989, Gold et al. 1991, Kelly et al. 1994). Thereafter, reports especially from Europe have described milder symptoms in these children and only rarely anaphylaxis (Michael et al. 1996, De Swert et al. 1997, Mazon et al. 1997, Bernardini et al. 1999). In the

present series, one reason for the mild symptoms in the multioperated children could be the low-allergen NRL gloves which were taken in use in our University Hospital as early as 1990 (Turjanmaa et al. 2000).

It is important to notice that one third of the present NRL-allergic children with a history of multiple operations were totally asymptomatic (I). This is in agreement with several previous studies of NRL-allergic children with spina bifida reporting that over half of the sensitized children did not present any symptoms (Michael et al. 1996, De Swert 1997, Bernardini et al. 1999). There is also one previous study which shows that even non-operated NRL-allergic children can be frequently asymptomatic (Liebke et al. 1996). Moreover, Novembre et al. (1997) reported that half of the 10 SPT-positive children had no clinical symptoms, and Bernadini et al. (1998) that none of the 8 SPT-positive school children had any history of symptoms. We as well as some other researchers (Liebke et al. 1996, De Swert 1997), however, have shown with the NRL glove use test that even these asymptomatic SPT-positive children can react to high-allergenic NRL gloves.

In summary, we could not find any major differences in the clinical findings between the present non-operated and multioperated children. Neither did the SPT reactivity to NRL allergens nor the mean IgE levels in the latex RAST differ between these two groups of children (I). This finding strongly suggests that the non-operated and the multioperated NRL-allergic children found by SPT screening have similar *in vivo* and *in vitro* reactivity to NRL allergens. However, larger patient groups and/or examinations with purified NRL allergens are needed to confirm whether some differences exist between these two groups of NRL-allergic children.

3. Frequencies of IgE antibodies to purified NRL allergens

The IgE antibody frequencies (see Results, Table 8) were rather similar in the non-operated and the multioperated children with regard to Hev b 6.02 (hevein) and Hev b 6.01 (prohevein) allergens. IgE antibodies to Hev b 6.01 were found in 86% of the non-operated children (II). Previously, Hev b 6.01 has been shown to be a major NRL allergen in NRL-allergic patients (Alenius et al 1996c, Banerjee et al. 1997). As expected, most (63%) of the present non-operated children had IgE antibodies also to Hev b 6.02 (II) which is the major IgE binding domain of prohevein (Alenius et al. 1996c). Similarly, 58% of the multioperated children had IgE antibodies to Hev b 6.01 and Hev b 6.02 (II). In agreement with this, Banerjee et al. (1997) showed IgE antibodies to recombinant Hev b 6.02 in 56% of 25 patients with spina bifida. The present finding of high frequency of IgE antibodies to Hev b 6.02 in both the non-operated and multioperated NRL-allergic children confirm that hevein is a major NRL allergen in both groups of children. Hevein is soluble in water and easily eluted from NRL gloves (Alenius et al. 1996c), which seems to be the reason for the frequent sensitization in children and adults exposed to NRL products.

The major difference in the IgE antibody frequency between the non-operated and the multioperated children was related to Hev b 1 and Hev b 5 (II, UR). Over 60% of the multioperated children had IgE antibodies to Hev b 1 and 33% to Hev b 5, whereas the frequencies in the non-operated children were significantly lower. The high IgE antibody frequency to Hev b 1 in the multioperated children agrees well with previous studies in which the frequencies were from 67% to 81% (Alenius et al. 1996a, Chen et

al. 1997b). The reason why the IgE antibody frequency to Hev b 1 is much lower in non-operated children and also in NRL-allergic adults is not known (Alenius et al. 1996a, Yeang et al. 1996). It has been speculated that this difference is due to different routes of sensitization and/or to more intense exposure to the NRL products. In children with spina bifida and other multioperated children, exposure to gloves and other medical NRL products often occurs on the mucous membranes during repeated operations and medical examinations. Hev b 1, and also Hev b 3, are known to be tightly bound to rubber particles, they are hydrophobic and therefore, seem not to elute easily from the gloves and other NRL products. In agreement with this, sensitization to Hev b 1 and Hev b 3 seem to occur first after frequent and/or prolonged mucosal exposure (Lu et al. 1995, Yeang et al. 1996, 1998, Chen et al. 1997b). It is of interest that also IgE antibodies to Hev b 5 were clearly more frequent (33% vs. 3%) in the multioperated than in the non-operated children. Previously, Slater et al. (1996) found by RAST that as many as 56% of the children with spina bifida had IgE antibodies to Hev b 5, but they did not examine the non-operated children.

Hev b 3 NRL allergen seems to sensitize mainly children with spina bifida and other children requiring multiple operations (Alenius et al. 1993, 1995b, Lu et al. 1995, Yeang et al. 1996). This 23 kD rubber particle allergen (previously described also as a 27 kD allergen) has marked structural homology with Hev b 1. Approximately 80% of the children with spina bifida and other children requiring multiple operations have IgE antibodies to Hev b 3 (Alenius et al. 1993, Lu et al. 1995). In the present study, we were unable to measure IgE antibodies to this allergen with ELISA, and with immunoblotting could show IgE binding to the 27 kD NRL allergen in only one of the 12 multioperated children (II). One explanation for the low frequency may be that Hev b 3 is broken into smaller peptides which would impair the detection of this allergen in immunoblotting (Lu et al. 1995, Yeang et al. 1996). One interesting aspect reported by Yeang et al. (1996) is a finding that Hev b 3 was fragmented upon addition of B-serum of crude NRL. This might indicate the presence of endogenous proteases which could have an effect on the allergen composition of NRL end-products.

IgE antibodies to Hev b 7, a patatin-like NRL allergen, were not found in any of the present NRL-allergic children (III). This shows that Hev b 7 is not an important allergen in these NRL-allergic children. This is in sharp contrast to NRL-allergic adults in the present and a previous study (Beezhold et al. 1994). With ELISA we found IgE antibodies to Hev b 7 in 49% and Beezhold et al. (1994) with immunoblotting in 23% of the NRL-allergic adults. Further studies are needed to clarify why NRL-allergic adults, but not children, have IgE antibodies to Hev b 7.

4. Casein in NRL gloves

In SPT screening with NRL glove extract, we found five children, all of whom were allergic to cow's milk, who at re-examination had a negative SPT to standardized NRL allergen (Stallergènes) and a negative latex RAST, but who still presented with a positive NRL glove use test (I). Because cow's milk casein can be added as a stabilizer during the glove manufacture (Subramaniam 1995), we suspected that the positive glove use test reactions could be due to casein eluting from this particular glove brand (Triflex). The casein content was measured by RAST inhibition and RIE. The mean content was about 400 µg/g of glove (IV). The casein content was further examined by RRIE in 30 brands of NRL gloves. Distinct amounts of casein were found in 8 brands

and minute amounts in 7 brands (IV). These findings confirm that casein eluting from NRL gloves could be responsible for the positive SPT and glove use test reactions in the cow's milk-allergic children. NRL glove extracts have been previously widely used by us and others in skin prick testing because of the lack of standardized SPT reagents. Moreover, the NRL glove use test has been shown to be a good method for confirming the diagnosis of NRL allergy in both children and adults (Turjanmaa et al. 1996, Liebke et al. 1996). To avoid false positive SPT reactions, a standardized reagent, now available in Europe, and waiting for the FDA approval in the USA (Turjanmaa et al. 1997, Hamilton et al. 1998), should be preferred in the screening and diagnosis of NRL allergy. When a use test is needed to confirm the allergy, it is necessary to know that the test gloves do not contain casein.

From a clinical point of view it is important to know that casein eluting from gloves can cause contact urticaria in children allergic to cow's milk. This allergy is common in children and may also, though rarely, be present in adults (Isolauri and Turjanmaa 1996, Sampson 1997). Previously, we reported an adult patient with allergy to cow's milk who experienced contact urticaria when using NRL gloves containing casein (Mäkinen-Kiljunen et al. 1993). We are, however, not aware of any patient showing systemic reactions from casein in NRL gloves. To avoid unexpected allergic reactions among persons allergic to cow's milk, casein should be labelled if it is used as an additive in NRL gloves. The best solution would be for the manufacturers to stop adding casein in NRL gloves. The gloves we examined for casein content were sampled from the market in 1994, and it would be interesting to know whether casein is still used today in the manufacture of gloves.

5. SPT reactivity to fruits and potato and IgE antibodies to Sol t 1 in NRL-allergic children

NRL-allergic patients frequently show allergic symptoms and/or positive SPT reactions to various fruits and vegetables, and the term "latex-fruit" syndrome has been suggested for this association (Blanco et al. 1994, Beezhold et al. 1996). SPT reactions to cross-reactive fruits were common also in the present NRL-allergic children. At diagnosis the frequencies were 87% in the non-operated and 50% in the multioperated children (I) No major change in SPT reactivity occurred during the follow-up (Table 9). In the present study we did not analyze the occurrence of clinical symptoms to the fruits or measure IgE antibodies to them. Previously, Brehler et al. (1997) found that 43% of the 136 NRL-allergic adults and Tücke et al. (1999) that 42% of the 12 children had clinical symptoms from the fruits, whereas Beezhold et al. (1996) reported that most of the NRL-allergic adults were asymptomatic. To clarify the clinical importance of the "latex-fruit" syndrome, carefully controlled challenge studies are needed in both the NRL-allergic children and adults.

Immunoblot inhibition studies have identified several cross-reactive allergens between NRL and various fruits such as banana, avocado and kiwi fruit (Ahlroth et al. 1995, Alenius et al. 1996b, Möller et al. 1998). Recently, these cross-reactive allergens in avocado, banana and chestnut have been identified as class I chitinases which have a N-terminal hevein-like domain (Mikkola et al. 1998, Blanco et al. 1999, Diaz-Perales et al. 1998, 1999, Posch et al. 1999, Sanchez-Monge et al. 1999). Interestingly, the present NRL-allergic children with positive SPT reactions to banana and other fruits frequently had IgE antibodies to hevein (Hev b 6.02), supporting the hypothesis that the observed

SPT reactions to the fruits could be cross-reactions between class I chitinases in the fruits and antihevein IgE antibodies.

In the present study, 63% of the NRL-allergic children showed a positive SPT to raw potato, which is in agreement with a previous study in adult patients (Beezhold et al. 1996). Sol t 1 is a main storage protein in potatoes and was recently shown to be a IgE binding allergen in atopic children with positive SPTs to raw potato (Seppälä et al. 1999). Since Sol t 1 and Hev b 7, a patatin-like NRL allergen, have a remarkable sequence homology (Beezhold et al. 1996, Kostyal et al. 1998), using ELISA we studied the prevalence of IgE antibodies to these allergens in the NRL-allergic children and adults. IgE antibodies to Sol t 1 were found in 66% of the NRL-allergic children and in 43% of the adults (III). In contrast to the frequent occurrence of IgE antibodies to Sol t 1, none of NRL-allergic children, but 49% of the adult patients, had IgE antibodies to Hev b 7 (III). The different IgE antibody prevalences and the results of ELISA inhibition studies performed in the adults suggest that several IgE epitopes could exist in Sol t 1, one which cross-reacts with Hev b 7 and another which does not. The IgE reactivity in the NRL-allergic children seems, therefore, to be restricted to the non-cross-reacting Sol t 1 epitopes. Recently, Tücke et al. (1999) examined by RAST inhibition cross-reactions of IgE antibodies to crude NRL and potato. In agreement with the present results, in one child they found evidence for co-sensitization for NRL and potato allergens. It is of interest that 30% of the present NRL-allergic children reacted to purified Sol t 1 in skin prick testing (III). This result suggests that the present NRL-allergic children could be clinically reactive to Sol t 1 in potato. Challenge studies are needed to confirm whether the NRL-allergic children with IgE antibodies and positive SPTs Sol t 1 get allergic symptoms from ingested potato.

6. Outcome of NRL-allergic children during follow-up

To study the outcome of NRL-allergic children we followed 32 children for a mean of 2.8 years. Two-thirds of the children could not totally avoid NRL contacts during the follow-up (V). This was rather surprising because at the time of diagnosis all the children and their parents had seen the contact urticaria symptoms from the glove use tests and had also been carefully advised by oral and written information on how to avoid NRL products. A third of the children experienced symptoms during the follow-up but these usually were mild. However, when blowing balloons two of the non-operated NRL-allergic children got a systemic reaction which required a visit to the emergency care (V). It seems, therefore, that more attention should be paid to protection of NRL-allergic children from NRL contacts in everyday life. In contrast to several allergic reactions experienced at home and in the everyday environment, 5 multioperated and two non-operated children underwent from 1 to 8 operations in hospital uneventfully (V). This good outcome was expected because the operations were performed with non-NRL gloves and other precautions suggested for NRL-allergic children (Charous et al. 1992, Meeropol et al. 1993).

During the follow-up, SPT reactivity to NRL allergens and IgE antibody levels in latex RAST or IgE ELISA did not show any marked decrease in the non-operated or multioperated children although they tried to avoid NRL contacts. Harrigan et al. (1999) measured IgE antibodies to NRL by ELISA in 16 children with spina bifida before and after institution of NRL precautions. In contrast to the present results, IgE levels to NRL decreased (at least one class) in 69% of the children. The reason for the discrepant

results could be a somewhat shorter follow-up time in our study or also continuous NRL exposure at home. Cremer et al. (1998) followed 67 children with spina bifida by measuring IgE antibodies to NRL with RAST after a latex-free environment had been established in the hospital. They found decreased IgE antibody levels in 49% and increased levels in 35% of the NRL-allergic children. Eight children with increased IgE antibody levels had had no additional operations, suggesting that NRL exposure could have continued at home in a similar way to that observed in the present study. A previous study in children allergic to cow's milk (Hill et al. 1993) showed that the SPT reactivity but not IgE levels decreased in those children who became clinically tolerant during the follow-up. It is evident that more research is needed to discover whether SPT reactivity or IgE levels to NRL or specific food allergens behave according to some general patterns during avoidance of these allergens in childhood.

Two previously asymptomatic, non-operated children experienced symptoms from balloons or household NRL gloves during the follow-up (V). This clearly shows that also previously asymptomatic but NRL glove challenge-positive children are at risk for NRL allergy symptoms, and thus they should carefully avoid NRL contacts at home environment. To analyze the outcome and sensitivity to NRL of the asymptomatic and symptomatic NRL-allergic children, we divided the 32 NRL-allergic children into two groups, i.e., those who had symptoms before the diagnosis (18 children) and those without (14 children), and compared these two groups with each other (V). In both groups the NRL contacts occurred during the follow-up with similar frequencies (72% and 64%, respectively), but the symptomatic children tended to experience symptoms more frequently (62%) than the asymptomatic ones (22%) (p=0.07). However, at the end of the follow-up the *in vivo* (SPT) and *in vitro* (latex RAST) reactivity did not differ between the symptomatic and asymptomatic groups (V). Our results, based mainly on the non-operated NRL-allergic children, do not support the findings of Mazon et al. (1997) and Bernardini et al. (1999) in children with spina bifida. They studied the risk factors of NRL allergy in this group of children and found that the symptomatic children were more likely to have positive SPTs and higher IgE antibody levels to NRL than the asymptomatic ones. One reason for the discrepant results could be that in the present study the NRL allergy diagnosis was confirmed with a NRL glove use test, whereas no challenges were performed in the studies of Mazon et al. (1997) and Bernardini et al. (1999).

NRL allergy is known to be associated with fruit and vegetable allergies (Blanco et al. 1994, Beezhold et al. 1996). Most of the present NRL-allergic children at diagnosis had positive SPTs to fruits such as banana, avocado and kiwi fruit, and no marked change occurred in the SPT reactivity during the follow-up (Table 9). We were not able to measure the IgE antibodies to these fruits with RAST nor challenge the children to these fruits. In contrast to NRL, no particular advice to avoid these fruits was given to the children and their parents. It can, therefore, be speculated that the children had been eating these fruits at least sometimes during the follow-up. This fruit exposure in the present children could also be one reason for the unaltered reactivity to NRL. Banana and avocado contain class I chitinases with a hevein-like domain, and these peptides are cross-reactive with the hevein in NRL (Mikkola et al. 1998, Blanco et al. 1999). Exposure to these cross-reactive fruits could, therefore, maintain IgE antibody levels and SPT reactivity to hevein and *vice versa*. Further studies are obviously needed to clarify whether exposure to the cross-reactive fruits could affect the outcome for NRL-allergic children.

7. Future aspects

We found a 1.7% frequency of positive SPTs when using NRL glove extract to screen 3,269 children for NRL allergy at our University Hospital in 1992-95. It seems that the prevalence of NRL allergy continues to be at the same level since SPT screening with Stallergènes allergen in 1996-98 still yielded positive results in 1-2% of the tested children (unpublished observation). In the present study, the diagnosis of NRL allergy was confirmed with strict criteria, i.e., by using SPT, latex RAST and glove use test, and the prevalence was then found to be 1%. Novembre et al. (1997) found a remarkably similar prevalence when they screened Italian children. The number of children in their study was a tenth of our study population. In both studies the children screened were mostly atopics admitted for inhalant or food allergy testing. Bernardini et al. (1998) screened a large population of unselected Italian school children for NRL allergy using SPT, and found positive SPTs in 0.7% of the children. To confirm this result in an unselected population, a similar study should be performed in Finland or some other country.

An important observation was that two-thirds of the present NRL-allergic children found in the screening did not belong to the well-known risk group of spina bifida or other multioperated children (Kelly et al. 1993, Nieto et al. 1996). Moreover, the clinical histories were often mild or even negative in both the non-operated and multioperated children, showing that screening with SPT is a valuable tool for detecting as many NRL-allergic children as possible. We included in-house glove and commercial NRL allergens in routine SPT series. This testing, now performed on over 5,000 children, has proved safe and has not caused any systemic reactions. The sensitivity and specificity of either SPT or latex RAST used in NRL allergy screening of children by us or others (Liebke et al. 1996, Michael et al. 1996, Mazon et al. 1997), needs a comment. False positive screening results seem to occur with a rather high frequency when the final diagnosis of NRL allergy is verified by a positive glove challenge test, as shown in the present and previously (Liebke et al. 1996). It seems that especially in children weak positive SPT reactions, i.e., smaller reactions than the histamine control, or low IgE levels to NRL in RAST, may not always be relevant for NRL allergy diagnosis. The commercial SPT reagent used in the present study in the final diagnosis of NRL allergy has been standardized immunologically and by skin prick testing in NRL-allergic adults but not in children (Turjanmaa et al. 1997). We used this reagent with obviously good results in the present follow-up study. It is obvious, however, that instead of using crude NRL allergen preparations or NRL glove extracts for screening and diagnostic purposes, better SPT and RAST reagents should be tailored in future using genetically engineered, recombinant allergens. These new reagents should then be evaluated in challenge proven series of NRL-allergic children and adults in order to minimize the occurrence of false positive and negative test reactions.

The fact that the present multioperated and non-operated children had mostly mild symptoms or were even asymptomatic was somewhat unexpected. Earlier studies of multioperated children have described frequently severe symptoms during surgical operations (Slater 1989, Gold et al. 1991, Nguyen et al. 1991, Kelly et al. 1993). In agreement with the results of the present study, recent reports have focused attention also on the presence of milder symptoms in NRL-allergic children (Michael et al. 1996, De Swert et al. 1997, Mazon et al. 1997). Our series of multioperated children was small, but another reason for the relatively mild symptoms in these children could be the

policy in our Hospital of using only low-allergen NRL gloves since 1990. Supporting this, our preliminary data have shown that this policy has already decreased the prevalence of NRL allergy among glove using employees in our Hospital (Turjanmaa et al. 2000). Whether the low-allergen glove policy would in the long run also lower the sensitization rate in children needing multiple operations should be evaluated in a prospective, comparative study in hospitals in which either a low-allergen or ordinary NRL glove policy is used.

Over half of the present NRL-allergic children showed positive SPTs to various fruits and potato which is an important issue. The cross-reactivity of NRL and fruit allergens such as avocado and banana has been characterized at the molecular level. Hevein (Hev b 6.02) and class I endochitinases in several fruits are structurally homologous and cross-reactive *in vitro,* and they can both also elicit positive SPT reactions in NRL-allergic patients (Mikkola et al. 1998, Diaz-Perales et al. 1998, Blanco et al. 1999). About 60% of the present non-operated and multioperated NRL-allergic children presented IgE antibodies to hevein and at the same time also had positive SPTs to various cross-reactive fruits. On the other hand, IgE antibodies to class I endochitinases in the fruits, which we did not examine, could also be one reason for weak and possibly false positive SPT reactions to NRL when screening children for NRL allergy. We noted that many of the present NRL-allergic children reacted in SPT to raw potato and also had IgE antibodies to potato patatin (Sol t 1). However, we could not find any evidence of cross-sensitization between Sol t 1 and Hev b 7 because none of the children had IgE antibodies to Hev b 7. The clinical importance of the frequent SPT reactivity to cross-reactive fruits and potato was not addressed in the present NRL-allergic children. Further studies, therefore, are needed to elucidate this aspect of NRL allergy. Even more important would be to investigate whether primary sensitization to class I endochitinases in the fruits at young age could result in a subsequent and clinically relevant NRL allergy in later life.

One prospective aspect for the treatment for NRL allergy is immunotherapy which might be used in future for both NRL-allergic children and adults. Knowledge of the major NRL allergens has increased considerably during recent years. The present and previous studies have shown that the presence of IgE antibodies to Hev b 1 (Alenius et al. 1996a, Chen et al. 1997b) and also to Hev b 5 (Akasawa et al. 1996, Slater et al. 1996) are good markers for NRL sensitisation related to multiple operations. The present study also revealed that Hev b 6.02, but not Hev b 7, is an important allergen for both multioperated and non-operated NRL-allergic children. The results for the non-operated children are novel and they should be confirmed by further studies. Exact knowledge of the relevant allergens in the NRL-allergic children and adults is essential for the production of better diagnostic reagents and also for the possible future development of immunotherapy.

G. SUMMARY

When we used NRL glove extract SPT to screen 3,269 children admitted for inhalant or food allergy testing and confirmed the diagnosis with a standardized NRL allergen SPT, latex RAST and glove use test, the prevalence of NRL allergy was found to be 1%. Although children with multiple operations are a well-known risk group for NRL allergy, two-thirds of the present 42 NRL-allergic children had no history of multiple operations. Moreover, a third of the NRL-allergic children had no history of symptoms though they had undergone operations in hospital or been in contact with balloons and other NRL products at home. Most of the non-operated (97%) and multioperated (83%) NRL-allergic children were atopics. These results show that routine skin prick testing, at present by using standardized reagents, is a valuable method of finding NRL-allergic subjects among children admitted for inhalant or food allergy testing. The glove use test is easy to perform and a high-allergenic glove brand can be recommended for use to confirm the clinical reactivity in the children who present with atypical or negative history of NRL allergy. It should be known, however, that certain NRL glove brands may contain moderate or minute amounts of cow´s milk casein. In addition to NRL allergic children we also found 5 children with cow´s milk allergy who presented with a "false" positive NRL glove use test reaction, i.e., contact urticaria, which was due to casein and not to NRL eluting from the gloves.

Allergological data from the non-operated, NRL-allergic children is scanty. Accordingly, we compared SPT reactivity to NRL and IgE antibody findings between the non-operated and multioperated NRL-allergic children. The findings in both groups of children were, however, similar with regard to SPT reactivity to glove eluate and commercial NRL allergen (Stallergènes), and IgE antibody levels in latex RAST. The IgE antibody frequencies and levels were also measured for the purified major NRL allergens using ELISA. Between 58% and 86% of the non-operated and multioperated children had IgE antibodies to Hev b 6.01 (prohevein) and 6.02 (hevein), which are therefore major NRL allergens in these two groups of children. The multioperated children significantly more frequently had IgE antibodies to Hev b 1 (rubber elongation factor, 67% vs. 27%) and Hev b 5 (33% vs. 3%) than the non-operated children, suggesting that IgE response to these two NRL allergens could be due to sensitization during multiple operations. None of the present NRL-allergic children had IgE antibodies to Hev b 7, a patatin-like NRL allergen, showing that this is not an important NRL allergen for children.

To examine the outcome of NRL allergy, 32 children were followed for a mean of 2.8 years. Despite careful instructions on avoidance of NRL, 22 (69%) NRL-allergic children were exposed to balloons and other NRL products at home. Ten (31%) children exhibited symptoms, 8 of them local contact symptoms and 2 children systemic symptoms. These results show that more attention should be paid to informing both non-operated and multioperated children and their parents on the risk of NRL allergy at home and in the everyday environment. The children followed did not show any change in SPT reactivity and IgE levels to NRL allergens, which supports an on-going NRL exposure in the home environment. On the other hand, the frequency of SPT reactivity to cross-reactive fruits (banana, avocado, kiwi fruit) as well as to potato remained high

during the follow-up. Continuous exposure of the NRL-allergic children to cross-reactive fruits and vegetables could possibly also maintain increased IgE levels to NRL allergens, and especially to hevein, because class I endochitinases in these fruits are known to contain a hevein-like domain.

In conclusion, the prevalence of NRL allergy is 1% among atopic children and it frequently affects also non-operated children. A third of the NRL-allergic children gave negative history of allergy symptoms. A combination of SPT with standardized allergen, latex RAST and NRL glove use test can be recommended for NRL allergy diagnosis in children. The major NRL allergens in children are hevein (Hev b 6.02), prohevein (Hev b 6.01) and also REF (Hev b 1) for multioperated children. During the follow-up two-thirds of the NRL-allergic children still had contacts with balloons, rubber boots or household gloves and could exhibit systemic or local symptoms, due to which the avoidance of NRL products in the home environment needs more attention.

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