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Immunoglobulin E that React with Natural Rubber Native Proteins in Thai Allergic Patient and Blood Donor Groups

การตรวจหาอิมมูโนโกลบูลิน อี ของผู้ป่วยภูมิแพ้และผู้บริจาคโลหิตที่ทำปฏิกริยาได้กับโปรตีนในน้ำยางธรรมชาติ

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การแพ้โปรตีนจากน้ำยางธรรมชาติมีรายงานออกมาอย่างมากมายในหลายประเทศรวมทั้งประเทศไทยซึ่งเป็นผู้ผลิตและส่งออกยางธรรมชาติรายใหญ่ของโลก เพื่อให้เกิดการตื่นตัวและระมัดระวังในความเสี่ยงที่จะเกิดการแพ้โปรตีนจากยางธรรมชาติในหมู่คนไทย การทดลองนี้ได้เปรียบเทียบปฏิกริยาระหว่างแอนติบอดีชนิด อี ในซีรัมของผู้ป่วยภูมิแพ้เด็กและผู้ใหญ่ กลุ่มละ 100 ราย เปรียบเทียบกับผู้บริจาคโลหิตจำนวน 352 ราย โดยใช้วิธี enzyme allergosorbent test (EAST) พบว่า ในกลุ่มผู้ป่วยภูมิแพ้ 200 ราย มีซีรัมที่ให้ผลบวก EAST กับโปรตีนจากน้ำยางธรรมชาติรวม 22 ราย หรือ ร้อยละ 11 ซึ่งสูงกว่าในกลุ่มผู้บริจาคโลหิตซึ่งมีเพียงร้อยละ 4.5 (16 ใน 352 ราย) อย่างมีนัยสำคัญทางสถิติ เมื่อคำนวณอัตราเสี่ยงของ ผู้ป่วยภูมิแพ้พบว่าสูงกว่าผู้บริจาคโลหิตถึง 2.6 เท่า ($p < 0.05$) การศึกษา immunoblot ยืนยันว่ามีโปรตีนอย่างน้อย 6 ชนิดที่ทำปฏิกริยากับแอนติ้บอดีชนิด อี ในซีรัมได้ ในกลุ่มผู้ป่วยภูมิแพ้ พบแอนติบอดีชนิด อี ต่อโปรตีนขนาด 30 kD ได้บ่อยที่สุด (12 จาก 22 ราย หรือร้อยละ 54) รองลงมาคือ 22.5 kD (พบ 7 ใน 22 ราย หรือร้อยละ 43) ในกลุ่มผู้บริจาคโลหิตพบแอนติบอดีต่อโปรตีนขนาด 25.5 kD บ่อยที่สุด พบ แอนติบอดีชนิด อี ต่อโปรตีนขนาด 30 kD เป็นอันดับสอง (7 ใน 16 ราย หรือร้อยละ 44 และ 5 ใน 16 ราย หรือร้อยละ 31 ตามลำดับ) จะเห็นว่าแอนติบอดีที่พบเป็นชนิด อี ซึ่งบ่งชี้ว่าน่าจะก่อให้เกิดอาการแพ้อย่างรุนแรงได้ เมื่อสัมผัสหรือได้รับโปรตีนเข้าไปโดยไม่ตั้งใจในคนไทยกลุ่มนี้ ในต่างประเทศมีรายงานว่าโปรตีนขนาด 14.6 และ 27 kD เป็นตัวก่อให้เกิดอาการแพ้ที่พบได้บ่อย ซึ่งต่างจากการทดลองนี้ ข้อมูลที่ได้จากการศึกษานี้คงช่วยกระตุ้นเตือนให้เกิดการดูแลควบคุมโปรตีนเหล่านี้ในกระบวนการควบคุมคุณภาพการผลิตผลิตภัณฑ์ยางธรรมชาติในอุตสาหกรรม

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นิรพนธ์ต้นฉบับ

Abstract : Kitiphong Harncharoen and Jariya Boonjawat. 2000. Immunoglobulin E that react with natural rubber native proteins in Thai allergic patient and blood donor groups. Thai J Hlth Resch 14(1) : 27-39.

Allergy to latex proteins has been reported extensively in several countries including in Thailand which is the top producer of natural rubber latex for export. For awareness of the risk of latex protein allergy in Thais, this study attempt to determine the allergic reaction between latex proteins and specific antibodies, IgE, in sera of atopic patients comprising of 200 adults and children, and 352 blood donor sera. A latex-specific enzyme allergosorbent test (EAST) is 11% positive with sera of atopic group, which is significantly higher than the control that give positive test only 4.5%. Statistic indicate that risk of rubber allergy in atopic patients is 2.6 fold higher than the normals ($p<0.05$). Immunoblot analysis confirm all the positive EAST test, and show that at least 6 bands of the fresh-field latex polypeptide bound to IgE in all the tested sera. The antibodies bound most frequently to a 30 kD (54%), follow by a 25.5 kD (43%) polypeptides in the sera with positive immunoblot of atopic group. For the control sera, the 25.5 kD (44%) and 30 kD (31%) polypeptides are frequently observed. Since these latex polypeptides are recognized by specific IgE-class antibodies, they are capable to be the major allergens in Thai population. Common allergens are recently reported among other countries, these suggest for the feasibility to use these protein antigens as indicators for a quality control of natural rubber products.

Key words : allergy, rubber, latex, blood donor, Thailand

Introduction

Natural rubber latex (NRL) is a complex cytosolic mixture obtained from the rubber tree *Hevea brasiliensis.* The latex is a major source of vitally all commercial natural rubber (cis-1,4-polyisoprene). Fresh NRL is preserved with ammonia, centrifuged, compounded and after aging, used in the manufacture of latex dipped articles. Products made from NRL include gloves, catheters, condoms, and hundreds of different medical and household devices. Recently, the medical community has become highly concerned with the increased latex allergy. Symptoms for this allergic reaction include urticaria, rhinitis, conjunctivitis asthma and less frequently, anaphylaxis, which is life-threatening (Slater *et al.*,1990; Tomazic *et al.*,1992; Slater and Trybul,1994). To date, several different high-risk groups have been identified, including hospital personnel, rubber industry workers, and children with spina bifida who have undergone multiple operations (Slater and Chhabra,1992). Universal precautions to prevent the spread of human immunodeficiency virus (HIV) have increased the frequency of latex exposure. Probably, repeated exposure to latex dipped articles together with genetic predisposition have led to latex sensitization of especially, atopic individuals. The first description of this IgE-mediated type I hypersensitivity to latex was reported by Nutter in 1979, and a number of IgE-reactive proteins in rubber raw materials and in processed rubber products have been identified (Slater and Trybul,1994; Slater and Chhabra,1992; Turjanmaa and Reunala,1990; Akaswa *et al.*,1993; Alenius *et al.*,1993; Kurup *et al.*,1993; Czuppon *et al.*,1993). On the other hand, type IV rubber sensitivity, manifested as contact dermatitis, has been documented, extensively in Europe and North America, mainly been ascribed to the chemical additives in rubber products (Wilson,1996; Lachapelle and Tenstedt,1979; Gonzales,1992). In Thailand, the contact dermatitis of rubber shoes has been reported by Jemesiriwatt since 1958. Research findings have shown that type I hypersensitivity source of sensitization appeared to be native proteins in the natural latex sap. A number of investigators have recently demonstrated that these native latex proteins are responsible for the observed allergic reactions to variety of latex products (Carrillo *et al.*, 1986; Morales,1989; Turjanmaa,1987). Four proteins (about 14, 20, 28 and 45 kD) in the clear serum (C-serum) of nonammoniated latex were shown to be reactive with sera of allergic children (Slater and Chhabra,1992). In 1993, Alenius *et al.* described six major allergenic proteins, of 30, 36, 39, 44, 46 and 54 kD, from a nonammoniated fresh latex. Sera from children with spina bifida reacted to a 14 kD protein in nonammoniated latex, and many of these sera also reacted to a 20 kD protein (Slater and Trybul,1994; Slater and Chhabra,1992). Czupon *et al.* in 1993 reported that the 14 kD protein was the major

allergen and similar to the rubber elongation factor (REF), which was found in a tetrameric form in latex. The N-terminal amino acids sequence were determined in a number of the latex allergens, such as 14 kD and 29 kD peptides were found to have sequence homologies to hevein and hevamine (Beezhold and Sussman,1994). A 27 kD allergen, eluted from latex gloves, was also demonstrated to be lysozyme by Yagami *et al.* in 1995.

The purpose of this study was to identify the prevalence of IgEs, which reacted to proteins precipitated from the C-serum of fresh nonammoniated latex in sera of atopic patients and blood donors and demonstrate the risk of NRL-allergy in Thai population. These specific IgEs, which have been used to be the immediate hypersensitivity index, will be used to identify the latex proteins which are potential allergen in Thailand, in order to develop a practical detection method for these latex protein allergens in the industrial NRL products.

Materials and methods

Patients and control subjects

Sera were obtained from 100 adults and 100 children with general allergic symptoms, whose age ranging from 18 to 65 years in adults and 6 to 12 years in children, from Prapinklao, Naval Hospital and Children Hospital in Bangkok. Three hundred fifty two control subjects were the blood donors of the Red Cross, Prapinklao Hospital Branch, with no history of latex allergy.

Preparation of latex proteins

The natural rubber field latex was collected from 4 clones of rubber tree, *Hevea brasiliensis*, (clone KRS 165 from Pong Rad Rubber Research Station, Chantaburi and clone RRIM 600, GT-1 and PB5/51 from plantations in Rayong Province, the Eastern part of Thailand). The fresh field latex samples were diluted with phosphate buffer (3:1 v/v) to the final concentration of 0.1 M phosphate, pH 7.4, and stored at $0^{\circ}C$ during transport and before processing in Bangkok. The latex specimens were centrifuged 20,000x g, 30 minutes, and proteins in the clear serum (C-serum) were precipitated by adding into cold-acetone at the ratio of 1:2, serum : acetone. The precipitated proteins were harvested by sedimentation, and resuspended in distilled water, and then dialyzed against water across a 1,000 Dalton molecular weight cutoff cellulose membrane overnight and lyophilized. The protein concentration was 0.44 g/g of field latex, as determined by Bradford protein assay (Bradford,1976) using bovine serum albumin (BSA) as standard.

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Enzyme allergosorbent test (EAST) (Czuppon *et al.*,1993)

The lyophilized latex proteins were dissolved in a sodium carbonate-bicarbonate buffer, 0.1 M, pH 9.6, at the final concentration of 5 g/ml. A polystyrene 96 well microtiter plate was coated with 200 µl/well of latex protein solution and incubated at 4° C overnight. After washing 3 times with 500 µl of 0.9% NaCl containing 0.1% Tween 20 (NSS/Tween 20) and blocked with 200 µl phosphate-buffered saline containing 3% BSA, 0.05% Tween 20 and 0.01% sodium azide (blocking buffer). Diluted serum (1:200, serum:NSS) of each atopic / control case 200 µl, was added to the wells and incubated at room temperature for 2 hr. Each well was washed 3 times with 500 µl NSS/Tween 20, then 100 µl goat anti-human IgE conjugated to alkaline phosphatase (Sigma Chemical Company, St.Louis, USA) in blocking buffer was added. After 2 hours of incubation at 37°C, the well was washed 3 times with NSS/Tween 20. Color product was developed by adding 100 µl of 0.1% p-nitrophenyl phosphate (Zymed Laboratories, San Francisco, USA) in 10 mM diethanolamine and 0.5 mM $MgCl_2$, pH 9.5. Absorbance reading at 405 nm was measured after 30 minutes of incubation at 37°C with an ELISA microplate reader (Titertek Multiskan, Labsystem and Flow Laboratory, Finland).

Immunoblotting (Slater *et al.*,1990)

The acetone-precipitated field latex proteins were separated on a 15 % gel sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in a mini-Protein II system (Bio-Rad Laboratories, Richmond, USA). The separated polypeptides were transferred to a nitrocellulose membrane (Micron Separations Inc., USA) at 240 mA for 2 hours in 15.6 mM Tris, 120 mM glycine buffer, pH 8.3. The membrane was cut into strips for immunostaining. Each strip was immersed in blocking buffer for 2 hours, and incubated at 4° C overnight in the diluted human EAST-positive sera (1:10), then washed in excess amount of NSS/Tween 20 and incubated in goat anti-human IgE conjugated to alkaline phosphatase for 2 hours at room temperature, then washed in excess amount of NSS/Tween 20. Color was developed with 20 ml 5-bromo-4-chloro-3-indolylphosphate and nitroblue tetrazolium (Zymed Laboratories, San Francisco, USA).

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Statistical analysis

The probability risk of the atopic patients and the control were calculated by case-control fourfold table (Mausner and Bahn,1974) and chi-square tests were determined for case / control risk factor by using a statistical software package, Microstat (Ecosoft Inc., USA).

Results

Samples of human serum in this study can be categorized into two groups, general allergic cases sera (200 cases)and blood donor control sera (352 samples). The allergic cases can be divided in three general allergic symptoms, 1) Ulticaria patients, 93 cases, 2) Rhinitis, 60 cases, and 3) Conjunctivitis, 47 cases (Table 1). These group are 37 men, 56 women, 56 boys and 44 girls. The control sera came from the healthy adults who came to donate blood regularly at the Red Cross and informed no history of any allergy. The control sera are 176 males and 176 females whose age ranging from 18 to 50 years old.

1. Prevalence of latex-specific IgE antibodies

Enzyme Allergosorbent Test showed positive results 22 cases (Table 1) or 11% in the allergic sera. In the control sera, there are 16 persons, 7 males and 9 females who showed positive EAST test (4.5%). The difference of prevalence between sex and age in each groups is not significantly different ($p > 0.05$).

2. Risk of NRL-allergy in Thailand

The probability risk of atopic patients to get anaphylactic hypersensitivity (3.989×10^{-3}) is statistically significant 2.6 fold higher than normal Thai population ($\chi^2 = 8.289$, $p < 0.05$) (Table 1) .

Table 1 Distribution of positive EAST results for anti-latex IgE in 552 atopic and control subjects by age and sex

Sample group	Sex (no.)	Age mean ± SD	Symptoms			Positive EAST (%)
			Ulticaria	Rhinitis	Conjunctivitis	
Atopic	Male	31.7 ± 7.3	22	8	7	4 (10.8)
Adult	(37)					
	Female	30.2 ± 6.7	34	19	10	8 (12.6)
	63)					
Children	Male	8.3 ± 5.1	20	18	18	6 (10.7)
	(56)					
	Female	7.6 ± 4.3	17	15	12	4 (9.1)
	(44)					
Total	200		93	60	47	22 (11.0)
Control						
	Male	37.5 ± 5.9	-	-	-	7 (3.9)
Blood donors	(176)					
	Female	29.8 ± 6.4	-	-	-	9 (5.1)
	(176)					
Total	352					16 (4.5)

3. Potential allergens in NRL-allergy.

The IgE antibodies from positive EAST sera bound in a heterogeneous manner to 6 polypeptides in NRL (Figure 1). The number of latex polypeptides that reacted with specific IgE in the sera of case and control group are varied, this study showed maximum 3 polypeptides can be found in one specimen (Figure1 and Table 2).The immunoblot test result was considered positive if the binding of IgE to at least one polypeptide was demonstrated. Positive immunoblot was detected most frequently against a 30 kD antigen which bound IgE antibodies in 12 of 22 (54.5%) patient sera, and 25.5 kD in 7 of 16 (43.8%) donor sera. Other antigens detected were 14, 18, 38 and 52 kD polypeptides (Table 3).

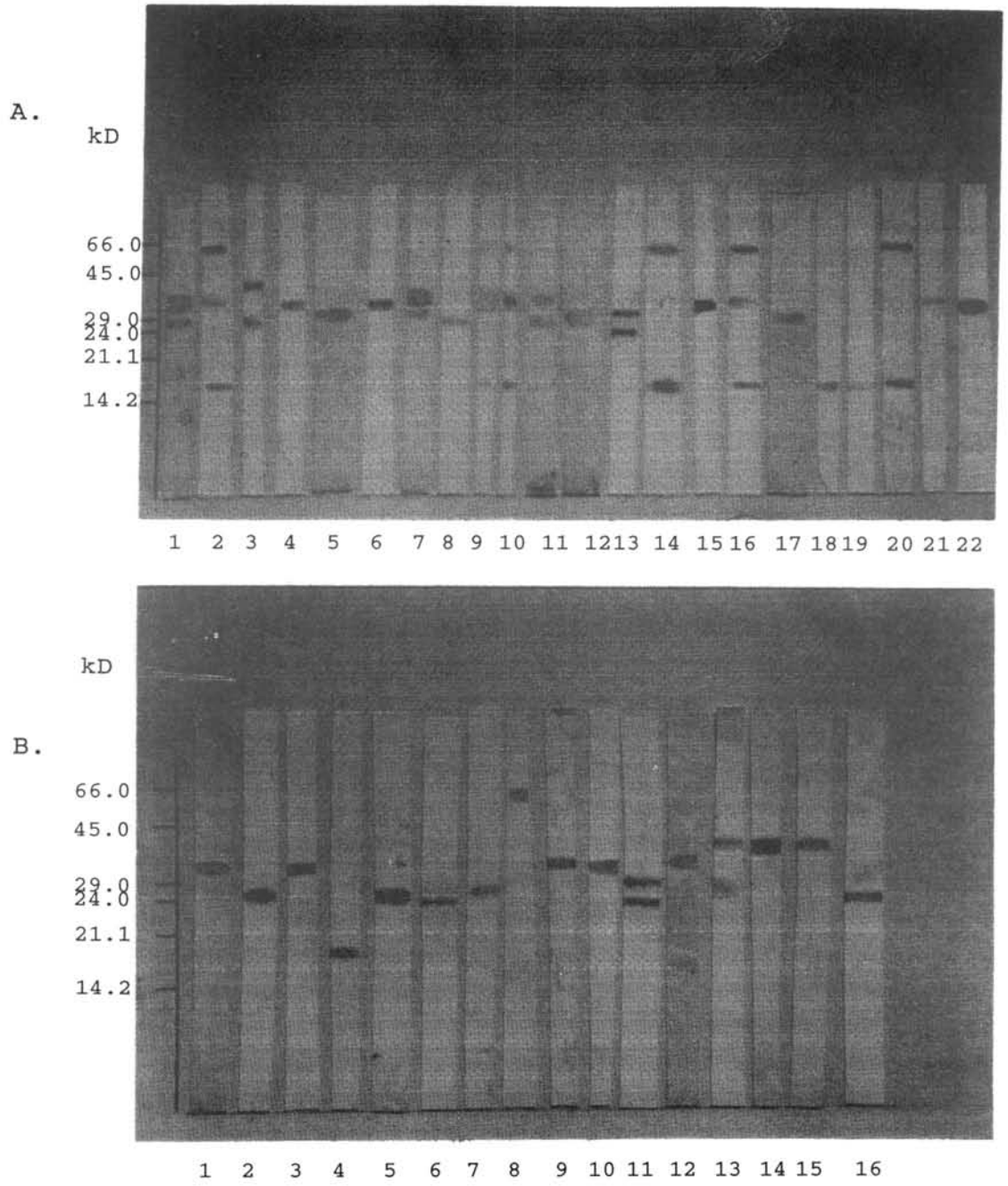


Figure 1 **IgE immunoblot of acetone precipitated tatex proteins with sera from atopic patients and control blood donors group which showed EAST positive results. A, Binding of specific IgE from sera of adult atopic case: strips 1-12, children sera strips 13-22. B, Specific binding of the IgE from blood donor control sera.**

Table 2 Number of latex protein antigens that react with the specific IgE in sera from atopic and control groups.

No. of latex bound to IgE	Atopic group	Control group
1	11	12
2	7	4
3	4	-
Total	22	16

Table 3 The frequency of latex protein antigens that react with the specific IgE in atopic and control sera

MW of latex protein antigen (kD)	Frequency of antigen reacted IgE (%)		
	Atopic sera	Control sera	Total
14	-	1/16 (6.2)	1/38 (2.6)
18	8/22 (36.4)	1/16 (6.2)	9/38 (23.7)
25.5	7/22 (31.8)	7/16 (43.8)	14/38 (36.8)
30	12/22 (54.5)	5/16 (31.2)	17/38 (44.7)
38	3/22 (13.6)	1/16 (6.2)	4/38 (10.5)
52	6/22 (27.3)	1/16 (6.2)	7/38 (18.4)

Discussion

In this study, comprising 200 atopic patient and 352 control sera as the source of IgE antibodies, we observed positive EAST test in both groups, which indicates prevalence of latex allergy. But the frequency of EAST test positive in atopic and control sera was different (22 patients (11.0%) and 16 patients (4.5%) respectively). The atopic patients are 100 adults and 100 children with the similar prevalence of the seropositivity for anti-latex IgE antibodies among the different age group and sex. Akasawa *et al.*(1993) reported the same rubber allergy prevalence, 11 from 108 atopic children by RAST in Japan using the same sample size as source of the IgE antibodies. Latex allergy in the lower age group (0.7-11.1 years) without any known risk factor could be very high as 72%, in a small sample group, 8 in 11 cases as reported by Sorva *et al.* (1995). Among the blood donor group, we cannot exclude the possibility that some of them may have been exposed directly to the latex such as previously been latex-farmer or rubber industrial worker because it was not possible to directly question the blood donors. But one of the four nurses, who were our donors, was

positive IgE antibodies. However, all these donor groups have no history of latex allergy or other allergy including these four nurses. The overall prevalence of positive EAST for the anti-latex antibodies among the donors obtained in this study is 4.5% which is more or less the same as 6.4%, reported by Ownby *et al.* (1996), in the study among 1,000 Red Cross volunteer blood donors in Southeastern Michigan.

These positive EAST samples also showed positive immunoblot test (38/38 or 100%). Previous studies has shown that avocado pear, banana and chestnut can cause cross-reactivity with latex by RAST (Makinen-Kiljunen,1994; Lavaud *et al.*,1995), but no reported about the cross-reactivity by EAST among latex and the fruits. We cannot comment on the relative diagnostic value of our test because we did not obtain the complete histories or skin test data on the blood donors. However, the positive results immunoblot test confirmed that the prevalence of our positive EAST results was the prevalence of anti-latex protein antigens.

This study found that, the IgE from positive sera, by EAST, could identify 6 antigenic rubber polypeptides (14, 18, 25.5 30, 38 and 52 kD) probably to be allergens in rubber IgE-mediated allergy. In previous studies by other investigators there are more than 15 natural rubber allergens detected in various rubber preparation, but only 10, 14, 20, 30 and 100 kD allergens have been demonstrated by more than one group (Slater and Chhabra,1992; Morales *et al.*,1989; Alenius *et al.*,1991; Chambeyron *et al.*,1992; Jaeger *et al.*,1992; Alenius *et al.*,1994). The 14 and 20 kD proteins had been shown to be the major allergens (Slater and Chhabra,1992). In the previous study, it seemed that the 30 kD antigen might be more significant than the 14 kD allergen (Chambeyron *et al.*,1992). This study also showed that the specific IgE for 30 kD protein was frequently found in the Thai sera. The 25.5 kD that found in this study also found in the report of Akasawa *et al.* (1995). Hev b 5, the acidic protein with molecular mass about 17 kD and homology to a protein from kiwi fruit, was recently reported that 52% of sera from patients allergic to latex express IgE reactivity (Slater *et al.*,1996; Akasawa *et al.*,1996). This protein has unusually high levels of glutamic acid, threonine, alanine, and proline residues and stains poorly with Coomassie blue on SDS-PAGE. Slater *et al.* Cloned and demonstrated that the recombinant peptide with a deduced molecular mass of 17.4 kD migrates at 36 kD rather than at the calculated mass. In the presence of 4 mol/L urea, the apparent molecular mass was reduced to 24 kD (Beezhold *et al.*,1999). This is a good example that molecular mass variation in biological molecules might be occurred

ลิขสิทธิ์มหาวิทยาลัย

Many cases of latex allergy and latex-induced intraoperatived anaphylaxis have been reported in Europe and the US but not in Thailand, the highest rubber production of the world. All of these general allergic cases did not realize that they were probably allergic to latex protein until we discovered it. This data suggest that there should be precaution about latex protein allergy for atopic patients if they were assigned for an operation or subjected to internal rubber-based medical devices such as catheter or other dental inserts.

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