Chapter 4

Discussion

4.1 Prevalence of the latex specific IgE

The overall prevalence of positive EAST for anti-latex IgE antibodies in 352 Red Cross blood 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 1,000 Red Cross volunteer blood donors in Southeastern Michigan. Among the blood donor group, there are four nurses which should be classified as high risk group, and one has positive IgE antibodies, however, all these donor group have no history of latex allergy or other allergy. The result of this study show that prevalence of specific IgE to latex protein antigens in 200 atopic patients is 11.0 % which is significantly higher than the control healthy blood donor group (4.5%). This result confirms that general allergy is one of the risk factor for latex allergy. These 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 , when about the same sample size was used 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).

These positive EAST results also showed positive results for immunoblot test (38/38 or 100%). Previous studies has shown that avocado pear, banana and chestnut can cause cross-reactivity with latex by RAST. It is possible that some of these EAST positive cases resulted from cross-reactivity between latex and some fruits like banana.

Althoug all positive EAST sera can react with at least one band of latex polypeptide in immuno blot, there was no cross-reactivity check with fruit proteins in this experiment. These EAST results can not be compared for the relative diagnostic value with other reports because there was no skin prick test, or RAST to compare with EAST and immunoblot.

4.2 Latex allergens

The study shows that, Thai people may develop IgE-mediated rubber allergy to more than one allergen in latex because the IgE positive sera reacted with 6 antigenic rubber polypeptides (14, 18, 25.5 30, 38 and 52 kD). In Finland, USA and other countries, more than 15 natural rubber protein allergens were report from various rubber protein preparation. Those of molecular weight 10, 14, 20, 30 and 100 kD allergens have been demonstrated by more than one group (Slater et al., 1994, Morales et al, 1989, Alenius, 1991, Chamberron et al., 1992, Jaeger et al., 1992 and Alenius et al., 1994). The 27 and 14.6 kD proteins had been purified and confirmed to be the major allergens in spina bifida children (Alenius et al., 1995), . Where the 30 kD was detected by Chamberron et al. (1992) and Turjanmaa et al. (1990) in latex and gloves extract. This study shows that the specific IgE for 30 kD protein is most frequently found in these Thai people. The 25.5 kD protein which was second to the 30 kD in frequency was also reported by Akasawa et al. (1995) in atopic children and hospital workers.

It is interesting to note that many reports describe latex allergen in the MW range of 27-29 kD. Lysozyme activity was detected with a latex allergen, of MW 27 kD protein (Yagami et al., 1995). This lysozyme has high homology with fruit lysozymes, and was suggested to be the relevance of defense-related proteins and cross-reaction of

latex allergy with fruit allergy, the 29 kD hevamine A and B described by Tata et al. (1993), of which their primary structure were determined by Jekel et al. (1991), and indicated homology of lysozyme from *H.brasiliensis* with pathogen-defense-related lysozyme from cucumber, fig, papaya and ranges of other plant lysozymes. Common antigenic determinant among those plant lysozyme has been suggested to be a cause of the cross reactivity between rubber latex and various fruits and these defense-related proteins could be markedly increased when a rubber tree was subject to wounding and phytohormone application like ethylene, commercially available in the form of Ethrel.

These data including this thesis suggest that there should be awareness and precaution about latex protein allergy in Thailand especially 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.

4.3 Immunoassay for latex allergen detection

In this study mixture of latex allergens 14-52 kD were used to raise allergen-specific rabbit antibodies. The advantage is to cover the whole range of potential allergens in fresh field latex, but the antibodies produced may give false positive result. The rabbit antibodies obtained do not cross-react with 5 non-latex proteins used to check for specificity of the rabbit IgG. All the three immunoassays based on the binding of antibodies to specific latex-protein antigens. The maximum titer obtained in four-rabbit sera were only 8-16, which resulted in moderate sensitivity (10 ng/well) of indirect competitive ELISA in the quantitative determination of latex allergens. When this immuno assay was used to quantitate allergens in latex from 4 rubber clones, the percentage of allergen were about 15-20 % of total protein. Quantification of latex allergen in 3 brans of examination gloves (Table 3.12) indicates that the amounts of

allergen are in the range of 18-20 % of total extracted, protein, which are approximately the same as the values observed in fresh latex from 4 rubber clones (15-20 % allergen/ total protein). Glove specimens from 3 different sources contain variable amount of total protein ranging from 90-420 μg/g, but the percentage of allergen determined by this ELISA method is very consistent, 18.6-20.1 % (Table 3.12) this result indicates that when total protein is known, the amount of allergen in glove can be estimated to be about 1/5 of total protein.

This estimation should be investigated in each type of NR product. In this study only glove and used rubber tires were investigated, but the content of allergen (5 μg/g specimen) in tires also fall in the same range as examination glove. Based on the assumption that rubber tire contains about 15 % NR, then the amount of allergen could be estimated as 34 μg/g NR, which fit well with the 18-85 μg/g range in examination gloves.

Swanson et al., (1994) used human IgE to quantify latex allergens. Beezhold et al., (1996) used anti-sera from rabbits (IgG) immunized with protein extract from ammoniated latex and develop indirect ELISA for latex protein concentration which showed good correlation with human IgE. The range of the assay was reported to be between 15-2,000 ng/ml, which is not better than this experiment (10-100 ng/well).

A modified Dot Blot technique developed in this thesis can eliminate the rubber background effect and useful for semi-quantitative analysis of the rubber sheet products. However, this method requires electro transfer equipment and need further development to be used as a quantitative method by using a refractrometer and modification of the transfer method and the immobilized medium that is cheaper than nitrocellulose.

A latex particle agglutination technique was initiated for the first time. An idea comes from the knowledge that proteins associated with the surface of latex particle are similar to soluble latex proteins in the serum. Major allergen such as rubber elongation factor, REF (Dennis and Light , 1989) with reported molecular weight of 14.6 kD which should be the same as 14 kD identified in this experiment (Figure 3.2 and Table 3.9.)

In the presence of latex-specific IgG, the glutaraldehyde-fixed latex particle (LP) formed agglutination rat that can be easily detected down to 3% DRC of LP concentration. Inhibition of agglutination is easily observed. The only limitation of this technique was the instability of the rubber particle after suspension glutaladehyde fixing. It has to be freshly prepared. The size of rubber particles were also variable with the diameter ranged from 250 to 800 nm depended on the period of preservation (Dennis and Light, 1989). This technique is useful for qualitative detection of latex allergen in the solution of protein extract from NR products.

The immunoassay developed in this study is still in the primary stage and not ready to be used in rubber industry. However, the overall results should be useful as basic information for further development of a diagnostic kit for detection of rubber latex allergens in NR product and investigation on current status of rubber allergy in Thai population.

Conclusion

The prevalence and risk of latex protein allergy in 352 Thai people investigated by EAST and immunoblot is 4.6 %. The general atopic group of 200 adults and children show relative risk 2.6 fold higher than normal healthy persons. Latex protein allergens that interact with specific human IgE are in the molecular weight range of 14-52 kD, where 30 kD and 25.5 kD proteins show the high frequency among 38 cases studied.

Three technique of immunoassays based on anti-latex allergens rabbit antisera were tested for their application with gloves and tires. An indirect competitive ELISA provides a consistent result between 10-100 ng/well of allergen was used to determine the percentage of allergen per total protein (μg/g) and amount of allergen (μg/g specimen) in two natural rubber products: gloves and used rubber tires. Specific quantitation immunoassay of latex allergen is evident by test with non-latex proteins and various sources of specimens. A modified dot blot and latex particles agglutination competition technique are not sensitive but specific to latex allergens. It is hoped that a simple latex particle agglutination competition can be further developed as a simple screening kit for rubber glove industry.