

Specific IgE antibodies in workers with occupational asthma due to western red cedar

K. S. TSE, H. CHAN and MOIRA CHAN-YEUNG

Department of Medicine, University of British Columbia, Vancouver General Hospital, Vancouver, B.C., Canada

(Received 27 July 1981; accepted for publication 3 November 1981)

Summary

The presence of specific IgE antibodies in the serum of patients with occupational asthma resulting from exposure to western red cedar (RCA) was studied by a radioallergosorbent test (RAST). The antigen matrix used in the RAST was either a conjugate of Sepharose particles with antigens in a crude cedar extract or with plicatic acid, the major haptenic component of cedar antigens. Of eighteen patients with clinical RCA and positive reaction to antigenic bronchoprovocation, eight were found to have abnormal RAST values. By appropriate absorption experiments, the serum RAST activity was shown to represent cedar antigen-specific IgE antibodies. No significant RAST activity was detected in the serum specimens from sixteen control subjects or from ten patients with negative bronchoprovocation-reaction to antigenic challenge. These results suggest that IgE antibody-mediated allergic reaction may be an important pathogenetic factor in RCA.

Introduction

Western red cedar is used commonly in British Columbia for both indoor and outdoor building constructions. Red cedar asthma (RCA) is the most common form of occupational asthma identified in this Province. In a previous survey (Chan-Yeung *et al.*, 1978), we have found that about 5.0% of the exposed population was affected with this occupational disease. The majority of the patients were cedar-mill, saw-mill workers or carpenters. There was usually a latent period of 3–4 years between initial exposure and onset of symptoms. Affected workers began to experience recurrent episodes of dyspnoea, wheezing and coughing. Initially these symptoms developed after the work shifts, but with continued exposure they might become symptomatic during the work shifts as well. About half of the patients complained of associated symptoms of rhinitis.

Antigenic bronchoprovocation is required for the positive confirmation of the

Correspondence: Dr Moira Chan-Yeung, Division of Respiratory Disease, Vancouver General Hospital, 686 West Broadway, 8th Floor, Vancouver, B.C., Canada.

clinical diagnosis. Three types of reactions to inhalation challenge have been observed (Chan-Yeung *et al.*, 1973). About half (45%) of the affected workers developed a late asthmatic response 4–6 hours after antigenic challenge, peaking in 6–10 hr and the bronchoconstrictive changes might last for a total of 24–48 hr. Immediate reaction alone, occurring in a few minutes and lasting up to 90 min, was observed only in a minority (about 7.0%) of affected workers. The remainder (about 48%) had a combined immediate and late, or dual reactions. Either a crude cedar extract or plicatic acid could be used in the bronchoprovocation test with similar results (Chan-Yeung *et al.*, 1973). Plicatic acid (PA) is a unique, small molecular weight (422 daltons) chemical compound present in western red cedar and its structural formula is shown in Fig. 1. Plicatic acid is a major haptenic component of the cedar antigens responsible for the asthmatic reaction.

Several of the clinical features of RCA strongly suggest the possible involvement of a hypersensitivity mechanism in its pathogenesis. Only a small proportion of the exposed workers developed this occupational asthma after a latent period of 3–4 years from the time of initial exposure. The clinical and pulmonary responses of the affected worker could be reproduced in the laboratory by antigenic bronchoprovocation with a small amount of either a crude cedar-extract or plicatic acid (adding further support to the hypersensitivity theory). In this report we demonstrate the presence of specific IgE antibodies in workers with RCA.

Materials and methods

Subjects

Twenty-eight workers suspected to have RCA were referred by their family physicians to the Respiratory Clinic of the University of British Columbia to undergo antigenic bronchoprovocation test in order to have their diagnosis confirmed. Of the twenty-eight patients, two had an immediate, nine had a late and seven had a dual (immediate and late) reaction following inhalation challenge with PA. Ten failed to react to antigenic challenge. The procedures for the bronchoprovocation test have been described previously (Chan-Yeung *et al.*, 1973). Serum specimens were taken from these twenty-eight patients as well as from sixteen control subjects, who had no history of occupational exposure to cedar, for the measurement of specific IgE antibody level.

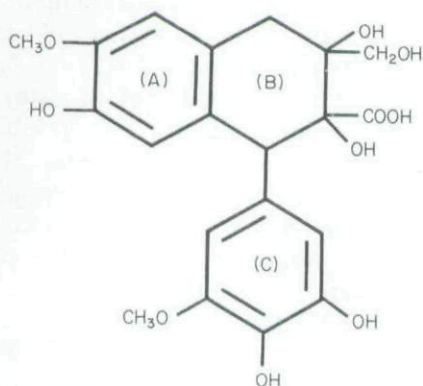


Fig. 1. Plicatic acid (mol. wt. 422 daltons)

We intentionally included seven subjects with known grass pollen allergy in the group of controls.

Preparation of crude cedar extract (CCE)

Red cedar-wood dust (10 g) was added to 100 ml phosphate-buffered saline (PBS, pH 7.4) and the mixture stirred at 4°C for 18 hr. The suspension was filtered through Whatman No. 1 filter paper and the filtrate spun at 15 000 rev/min for 10 min to remove the residues. The supernatant was used as the CCE which appeared to contain mostly small molecular weight substances. When the CCE was dialyzed against PBS at 4°C overnight, only less than 2.0% of the materials remained in the retentate, as determined by optical density readings of the extract before and after dialysis at 280 nm. The dialysis tubing used was Spectrapor (Fisher Scientific Co., Canada) which should retain compounds with molecular weight larger than 6000–8000 daltons. Similarly, when 2 ml of CCE was passed through a 2.6 × 90 cm column containing Sephadex G-50 (Pharmacia Inc., Sweden), the three peaks in the elution profile (Fig. 2) corresponded to the molecular weights of 1200, 600 and 422 daltons respectively. The last and major peak represents PA.

Conjugation of CCE to Sepharose-cl-4B

Two grams of cyanogen bromide were added to 40 ml Sepharose-cl-4B (Pharmacia Inc.), suspended in 40 ml of distilled water. The suspension was stirred continuously at room temperature. The pH of the suspension was adjusted to 11.0 by the addition of 1 N sodium hydroxide. The reaction was completed when the pH of the solution remained steady at 10.0 in about 15 min. The suspension was filtered through a Buchner funnel, washed thoroughly with cold distilled water and rinsed twice with cold bicarbonate buffer (0.1 M, pH 8.6). Ten millilitres of CCE were added to the activated Sepharose and the suspension stirred at 4°C overnight. The coupled Sepharose was washed a few times with 0.1 M bicarbonate buffer and suspended in 20 ml of 0.5 M

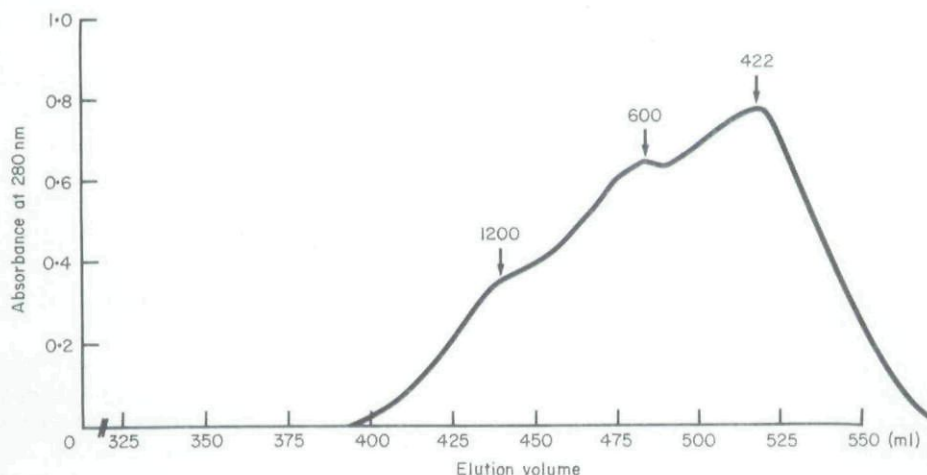


Fig. 2. The elution profile of 2 ml of CCE through a Sephadex G-50 column, 2.6 × 90 cm. The molecular weights corresponding to the three peaks are 1200, 600 and 422 daltons respectively. The molecular weight calibrations were based on the elution profiles of three substances on this column, namely ovalbumin (45 000 daltons), ribonuclease (12 500 daltons) and PA (422 daltons).

ethanolamine (in 0.1 M bicarbonate buffer, pH 8.6) (Sigma Chemical Co., U.S.A.) for 2 hr at 4°C. The final product was washed twice with 0.1 M bicarbonate buffer, once with 0.1 M acetate buffer (pH 4.0) and twice with PBS (pH 7.4). Then, it was stored as a suspension in RAST buffer (Pharmacia Inc.).

Conjugation of PA to human-serum albumin (HSA)

Plicatic acid (422 mg or 1 mM) was stirred slowly until dissolved in a mixture of 15 ml dioxane (Fisher Scientific Co.) and 2 ml of distilled water. Then 0.475 ml tributylamine (Eastman Kodak Co., U.S.A.) was added to the above solution followed by 0.11 ml isobutylchloroformate (Sigma Chemical Co.). The solution was stirred for 30 min at 4°C. Then 1.15 g of HSA was dissolved in 30 ml distilled water to which 1 ml of 1 N sodium hydroxide was added. The HSA and PA solutions were mixed and the mixture was stirred at 4°C for 4 hr. The organic layer was removed, as much as possible, and the solution was dialyzed against PBS for 24 hr, changing the buffer twice. Then a 4 ml aliquot was passed through a 2.6 × 90 cm column containing Sephadex G-100 (Pharmacia Inc.) to separate the conjugated PA-HSA from unconjugated free PA which had a distinct brown colour. The portion containing the PA-HSA conjugates also had a brownish tinge. By using tritiated Pa in parallel experiments, the number of PA groups that could be attached to a molecule of HSA was estimated to vary between eight and fourteen by this conjugation procedure.

Conjugation of PA-HSA, anti-IgE and of anti-IgG to Sepharose-cl-4B

The coupling procedures were the same as that used for the CCE-Sepharose conjugates as described earlier. The ratio of PA-HSA to Sepharose used in the reaction mixture was 1 mg/ml activated Sepharose. Epsilon chain-specific anti-IgE and gamma chain-specific anti-IgG were purchased from Kallestad Inc., U.S.A. A ratio of 2 mg of the antiserum to 1 ml of activated Sepharose was used in the conjugation procedure.

RAST

Each test tube had 0.05 ml suspension of (PA-HSA)-Sepharose or CCE-Sepharose, followed by 0.05 ml of test serum, pipetted into it. The tubes, all in duplicate, were shaken on a clinical rotator for 3 hr at room temperature, washed four times with RAST washing-buffer and were filled with 0.05 ml of ¹²⁵I-labelled rabbit anti-human IgE (Pharmacia Inc.) The tubes were again shaken on a clinical rotator at room temperature overnight and washed four times with RAST washing-buffer. The radioactivity in each tube was measured by a gamma counter. The average radioactivity in the duplicate tubes containing PBS, instead of serum, served as the baseline. The RAST values of the sera specimens were expressed as the multiples of this baseline radioactivity in counts per minute.

The specificity of the RAST activity in the patients' sera was determined by comparing the effects of prior absorption of the test serum with either anti-IgE or anti-IgG. Serum specimens (0.25 ml) were absorbed with 0.5 ml of either anti-IgE-Sepharose or anti-IgG-Sepharose and the RAST activity of the absorbed sera were then compared with that of the non-absorbed specimens. Furthermore, the antigenic specificity of the serum RAST activity was also determined by the degree of inhibition of RAST value that could be observed by prior absorption of the serum specimens with either CCE-Sepharose or (PA-HSA)-Sepharose.

Results

RAST values

The results of the serum RAST values of the twenty-eight patients who were clinically suspected to have possible RCA are shown in Table 1 and Fig. 3. The mean RAST values of the group of sixteen controls which included seven subjects with grass pollen allergy was 1.16 (for RAST activity with specificity for PA) and 0.96 (for RAST activity with specificity for the crude cedar-antigens). We chose an arbitrary number of 2 as a cut-off point for abnormal RAST values which represented a value greater than the mean plus 2 s.d. from the group of controls. None of the ten patients with negative reaction to antigenic bronchoprovocation had an abnormal RAST value. Of the eighteen patients with positive antigenic-inhalation tests, eight (44.4%) had abnormal RAST values. In all these eight cases, the RAST activity specific for PA paralleled the RAST activity specific for the crude cedar-antigens. One of these eight patients had an immediate bronchial reaction to antigenic bronchoprovocation, three had a late reaction and four had dual reactions.

The differences in the RAST values (both anti-PA and anti-CCE) between the group of eighteen patients who reacted positively to antigenic bronchoprovocation and the group of ten patients with negative bronchoprovocation tests were statistically significant ($P < 0.05$) when examined by analysis of variance. Similarly, the differences in the RAST values between the group with positive bronchoprovocation response and controls were also statistically significant.

Specificity of the serum RAST activity (antibody class)

Sera from three patients with elevated RAST values were absorbed with anti-IgE and anti-IgG respectively. As can be seen from the results shown in Table 2, absorption of the sera with anti-IgE but not with anti-IgG abolished the RAST activity.

Specificity of serum RAST activity (antigen)

Removal of the serum IgE antibodies with specificity for PA by absorption with different amounts of solid-phase (PA-HSA)-Sephacrose resulted in a dose-dependent inhibition of serum anti-PA, RAST values. The results are shown in Fig. 4 which represented the sera from four patients with high RAST activity. Similarly, absorption with CCE-Sephacrose also resulted in a dose-dependent inhibition of anti-CCE serum RAST values. The results on the study of three sera were shown in Fig. 5.

Among the group of sixteen controls, we included seven subjects with definite clinical manifestations of grass pollen allergy. All these seven sera had very high anti-grass pollen RAST activity but the anti-PA and anti-cedar RAST values of these sera were all normal (Table 3). This would serve as another test of the antigenic specificity of the anti-cedar and anti-PA RAST.

Discussion

Several of the clinical features of RCA strongly suggest that this type of occupational asthma might involve a hypersensitivity mechanism in its pathogenesis. Results of our previous studies indicated that only a small portion (about 5.0%) of the exposed workers developed RCA after a latent period of 3–4 years (Chan-Yeung *et al.*, 1978). The clinical and pulmonary reactions could be reproduced by inhalation challenge with a small amount of cedar antigens (Chan-Yeung *et al.*, 1973). Using the standard

Table 1. Serum RAST values obtained using two different antigenic matrices, PA-HSA and CCE, in the RAST

Subjects	n	Mean $\pm$ s.d.	PA-HSA		Crude cedar-extract	
			No. with abnormal values*	Mean $\pm$ s.d.	No. with abnormal values*	
Controls	16	1.16 $\pm$ 0.32†	0	0.96 $\pm$ 0.21†	0	
Patients reacted negatively to antigenic bronchoprovocation	10	1.10 $\pm$ 0.23	0	1.33 $\pm$ 0.21	0	
Patients with positive inhalation challenge						
Late reaction	9	2.48 $\pm$ 2.00	4	3.09 $\pm$ 3.15	4	
Immediate or dual reaction	9	3.45 $\pm$ 3.78	4	3.68 $\pm$ 3.85	4	

* RAST value greater than 2 was regarded as abnormal.

† The radioactivity count of a PBS control-tube without serum was used as the baseline which had a RAST value of 1.

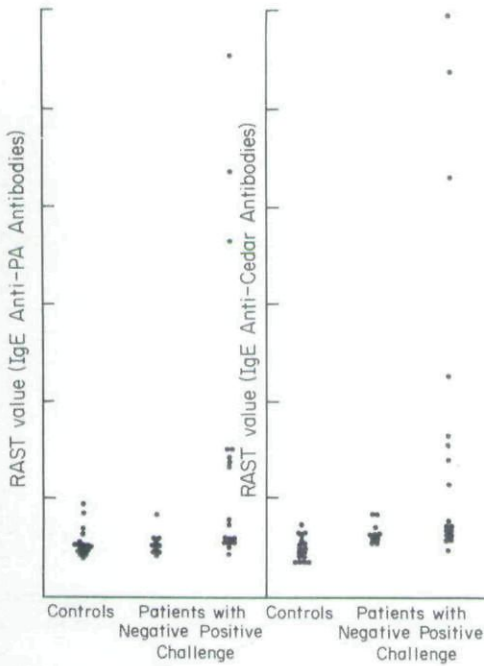


Fig. 3. Serum RAST values of the three groups of subjects as described also in Table 1.

Table 2. Effects of absorption by anti-IgE and anti-IgG on the serum RAST-value

Patient	Serum anti-PA RAST value			Serum anti-CCE RAST value		
	Before absorption	After absorption with anti-IgE	After absorption with anti-IgG	Before absorption	After absorption with anti-IgE	After absorption with anti-IgG
K.D.	8.77	0.94	8.41	8.68	1.09	8.58
J.D.	8.47	1.04	8.29	9.79	1.06	9.36
J.S.	5.46	0.95	5.37	3.16	0.93	3.05

RAST, we were able to demonstrate the presence of specific IgE-antibodies in about half of the patients with a clinical diagnosis of RCA, who also reacted positively to antigenic bronchoprovocation. Such IgE antibodies were not found in the group of patients with negative response to bronchoprovocation or in the group of control subjects. These results would lend support to the clinical impression that IgE antibody-mediated allergic reaction might be an important contributing pathogenic factor in RCA.

The specificity of the IgE antibodies was shown to be directed against the antigens contained in CCE as well as against the special small molecular weight hapten of western red cedar, PA. Studies of the CCE by dialysis in spectapore tubing and by Sephadex G-50 column chromatography revealed that the bulk of soluble materials in the CCE appeared to be low molecular weight substances (less than 2000 daltons) and PA appeared to be the major hapten of CCE. Clinically, positive bronchoprovocation

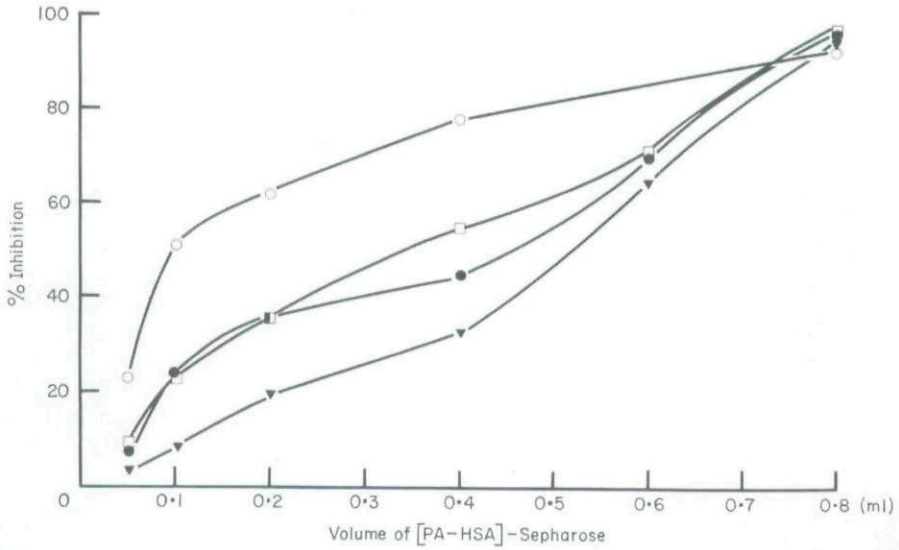


Fig. 4. Changes in the serum anti-PA, RAST values when 0.25 ml of serum was absorbed with varying volumes of (PA-HSA)-Sephadex conjugates. The four lines represent the serum obtained from four different patients with high RAST-titres (○, JS.; □, K.D.; ●, C.U.; ▼, J.D.). The degree of reduction in serum RAST values after absorption is expressed as:

$$\% \text{ inhibition} = \frac{\text{RAST value of (serum absorbed with HSA-Sephadex - serum absorbed with PA-HSA-Sephadex)}}{\text{RAST value of serum absorbed with HSA-Sephadex}} \times 100$$

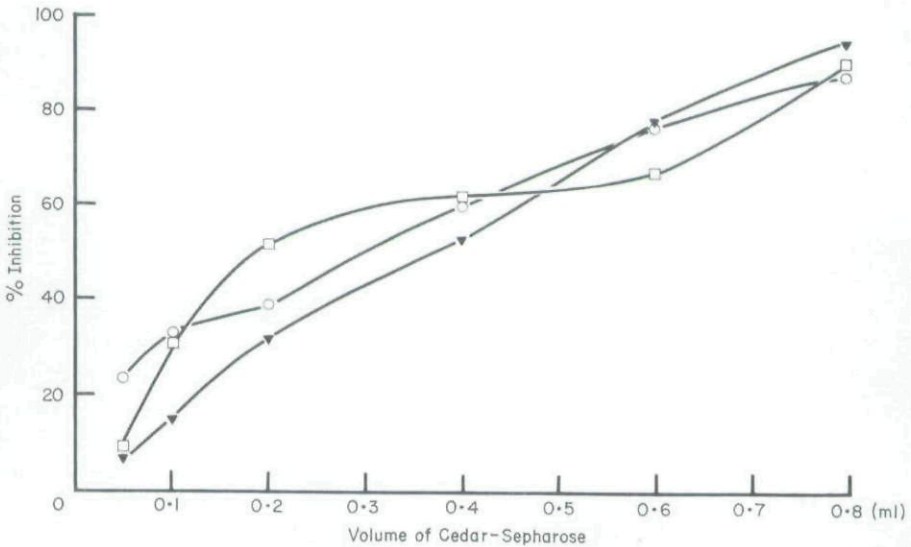


Fig. 5. Reduction of serum RAST values after 0.25 ml of sera from three patients with high RAST titres were absorbed with varying amounts of CCE-Sephadex conjugates. The calculations were the same as described in the legend of Fig. 4, substituting CCE-Sephadex for PA-HSA-Sephadex. ○, JS.; □, K.D.; ▼, J.D.

Table 3. Serum RAST value of seven controls with grass pollen allergy

Controls	Antigen matrix used in RAST		
	(PA-HSA)-Sepharose	CCE-Sepharose	Grass pollen extract-Sepharose
K.T.	1.07	0.97	40.6
T.M.	1.40	1.18	27.6
J.S.	1.04	1.33	23.3
P.I.	1.45	1.26	17.5
L.A.	1.16	1.09	9.0
P.S.	1.10	0.98	6.6
K.N.	1.03	0.84	3.9

could be elicited as effectively by inhalation challenge of sensitive workers with PA as with CCE.

Taken together, these results indicate that RCA represents another example of occupational asthma associated with exposure to low molecular weight compounds and anti-PA IgE antibodies may play an important pathogenetic role in the chain of events leading to the development of clinical RCA.

The presence of specific IgE anti-hapten antibodies had also been reported in other cases of occupational asthma, associated with exposure to low molecular compounds such as toluene diisocyanate (TDI) (Butcher *et al.*, 1980; Butcher, *et al.*, 1977) diphenylmethane diisocyanate (Zeiss *et al.*, 1980), phthalic anhydride (Maccia *et al.*, 1976) and trimellitic anhydride (Zeiss *et al.*, 1977). The pathogenesis of this group of occupational asthmas probably involves complex mechanism in addition to the possible IgE antibody-mediated reactions. Workers with occupational asthma related to exposure to cedar or TDI share one common characteristic with other asthmatics in general, i.e. the presence of non-specific bronchial hyper-reactivity (Butcher *et al.*, 1977; Butcher *et al.*, 1979; Chan-Yeung, 1977). The exact mechanism leading to the development of bronchial hyper-reactivity in occupational asthma remains to be elucidated. In the case of TDI-asthma, alteration of the function of the β -adrenergic receptor has been proposed as a possible mechanism as it has been shown that TDI inhibited the rise in lymphocyte cyclic 3' 5' adenosine monophosphate level following stimulation with either isoproterenol or prostaglandin (Butcher *et al.*, 1979; Davies *et al.*, 1977).

The ability of PA, a small molecular weight compound, to induce a positive inhalation-challenge response in sensitive workers would indicate that PA could react rapidly with certain body proteins, after inhalation, to form a complete allergen. The likely site of linkage with body proteins is via the catechol ring of PA (Fig. 1, ring C). However, skin testing of cedar workers with PA was unable to elicit any significant weal and flare reaction in patients with RCA. This discrepancy between the bronchial reactivity and dermal non-reactivity to PA in sensitive cedar-workers is unclear. One possible explanation could be that the appropriate carrier-proteins for PA to form a complete antigen are present in the bronchial, but not in the dermal tissues.

The site of the chemical linkage between PA and the carrier protein could influence which of the three rings' structures of PA may function as relevant antigenic-determinant for the induction of, and later on reaction with the specific IgE antibodies in

sensitive cedar-workers. In the present study, a PA-HSA conjugate was used in the RAST for the detection of specific anti-PA IgE antibodies. The mixed-anhydride method that we used to prepare the conjugates would link the carboxyl group in the 'B' ring of PA (Fig. 1) to HSA by the formation of an amide bond. The antigenic determinants of PA that are exposed in these conjugates may be quite different from that in the PA-carrier protein conjugates formed in the bronchial tissues of cedar workers from actual industrial exposure. This might be one reason to explain the relatively low RAST titre of anti-PA antibodies (as compared with the RAST titres of grass pollen-allergen sera) described in the present study. We plan to carry out further studies in the near future, using different types of PA-protein conjugates for the RAST assay. We wish to determine if conjugates with different configurations of the PA antigenic determinants will be able to reveal higher RAST titres of anti-PA antibodies and, therefore, to demonstrate the presence of specific IgE anti-PA antibodies in a higher portion of the cedar-sensitive workers. A comparable situation was reported in a recent study of the antigenicity of TDI (Karol, 1980). Antibodies obtained from guinea pigs sensitized by inhalations of TDI were found to have different degrees of reactivity with TDI hapten as compared with three forms of tolyl monoisocyanate monomers. The differences in sensitivity were likely the results of the variations in the antigenic determinants on the various forms of TDI and tolyl haptens.

Acknowledgments

This study was supported by a grant from the Medical Research Council of Canada.

References

- BUTCHER, B.T., JONES, R.N., O'NEIL, C.E., GLINDMEYER, H.W., DIEM, J.E., DHARMARAJAN, V., WEILL, H. & SALVAGGIO, J.E. (1977) Longitudinal study of workers employed in the manufacture of toluene-diisocyanate. *American Review of Respiratory Diseases*, **116**, 411.
- BUTCHER, B.T., KARR, R.M., O'NEIL, C.E., WILSON, M.R., DHARMARAJAN, V., SALVAGGIO, J.E. & WEILL, H. (1979) Inhalation challenge and pharmacologic studies of toluene diisocyanate-sensitive workers. *Journal of Allergy and Clinical Immunology*, **64**, 146.
- BUTCHER, B.T., O'NEIL, C.E., REED, M.A. & SALVAGGIO, J.E. (1980) Radioallergosorbent testing of toluene diisocyanate-reactive individuals using p-tolyl isocyanate antigen. *Journal of Allergy and Clinical Immunology*, **66**, 213.
- CHAN-YEUNG, M., BARTON, G., MACLEAN, L. & GRZYBOWSKI, S. (1973) Occupational asthma and rhinitis due to red cedar. *American Review of Respiratory Diseases*, **108**, 1014.
- CHAN-YEUNG, M. (1977) Fate of occupational asthma; a follow-up study of patients with occupational asthma due to western red cedar. *American Review of Respiratory Diseases*, **116**, 1023.
- CHAN-YEUNG, M., ASHLEY, M.J., COREY, P., WILLSON, G., DORKEN, E. & GRZYBOWSKI, S. (1978) A respiratory survey of cedar-mill workers. I. Prevalence of symptoms and pulmonary function abnormalities. *Journal of Occupational Medicine*, **20**, 323.
- DAVIES, R.J., BUTCHER, B.T., O'NEIL, C.E. & SALVAGGIO, J.E. (1977) The in-vitro effect of toluene diisocyanate in lymphocyte cyclic adenosine monophosphate production by isoproterenol, prostaglandin and histamine. *Journal of Allergy and Clinical Immunology*, **60**, 223.
- KAROL M.H. (1980) Study of guinea pig and human antibodies to toluene diisocyanate. *American Review of Respiratory Diseases*, **122**, 965.
- MACCIA, C.A., BERNSTEIN, I.L., EMMETT, E.A. & BROOKS, S.M. (1976) In vitro demonstration of specific IgE in phthalic anhydride hypersensitivity. *American Review of Respiratory Diseases*, **113**, 701.
- ZEISS, C.R., KANELAKES, T.M., BELLONE, J.D., LEVITZ, D., PRUZANSKI, J.J. & PATTERSON, R. (1980) Immunoglobulin E-mediated asthma and hypersensitivity pneumonitis with precipitating anti-hapten antibodies due to diphenylmethane diisocyanate exposure. *Journal of Allergy and Clinical Immunology*, **65**, 346.
- ZEISS, C.R., PATTERSON, R., PRUZANSKI, J.J., MILLER, M.M., ROSENBERG, M. & LEVITZ, D. (1977) Trimellitic anhydride-induced airway syndromes: clinical and immunologic studies. *Journal of Allergy and Clinical Immunology*, **60**, 96.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.