

Toluene-diisocyanate induced asthma: evaluation of antibodies in the serum of affected workers against a tolyl mono-isocyanate protein conjugate

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Summary

Radioallergosorbent testing was used to look for the presence of specific IgE antibody against a para-tolyl-mono-isocyanate human serum albumin conjugate in sera from five groups of subjects. The first three groups consisted of individuals exposed to toluene diisocyanate (TDI) who had been shown by bronchial provocation testing with levels of TDI below the threshold limit value of 0.02 parts/10⁶, to have immediate asthmatic reactions, late asthmatic reactions or no respiratory changes at all. The two control groups consisted of atopic and non-atopic individuals who had no respiratory symptoms and no known exposure to TDI. Although RAST showed high ct/min in some of the sera from patients with proven TDI-induced respiratory disease, these levels were not significantly different from controls and appeared to reflect the presence in these sera of high levels of total IgE ($> 100 \text{ u ml}^{-1}$). There is no evidence from this study for the presence of specific IgE antibody against a para-tolyl mono isocyanate human serum albumin conjugate in patients with TDI-induced respiratory disease. This finding may reflect absence of antibodies, or that the techniques for their detection are not always effective even when performed by experienced persons, and there is a potential source of error in the interpretation of results when sera contain large amounts of IgE.

Introduction

Toluene diisocyanate (TDI), a highly reactive low molecular weight chemical, forms a polyurethane when reacted with a polyol. This reaction is exothermic and generates sufficient heat to vaporize unreacted TDI. Those working in processes in which polyurethanes are produced may be exposed to TDI vapour and about 5% develop asthma (Butcher *et al.*, 1979). Bronchial airflow obstruction can be provoked in these individuals by exposure to atmospheric concentrations of TDI well below the current threshold limit value of 0.02 parts/10⁶. However the pathogenesis of these asthmatic reactions remains unclear. Davies *et al.* (1977) demonstrated that TDI has beta adrenoceptor blocking activity and suggested that this might play an important role.

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Alternatively there is some evidence that type I allergic mechanisms may be involved (Munn, 1965). In 1970, Taylor reported the finding of specific reaginic antibody against TDI in the sera of affected workers, but this has not been confirmed (Butcher *et al.*, 1977).

Recently specific IgE antibodies have been reported in the serum of symptomatic workers exposed to TDI, against a para-tolyl-mono-isocyanate (*p*-TMI) protein conjugate. It was suggested that the use of *p*-TMI with only one reactive isocyanate group, to prepare hapten-protein conjugates, instead of TDI which has two reactive isocyanate groups, would prevent cross linking, and increase the likelihood of demonstrating specific IgE antibody against the tolyl group. Further, this conjugate could be characterized spectroscopically (Karol *et al.*, 1978a,b).

In this study we have used two separate methods, including that of Karol *et al.* (1978a,b) to look for specific IgE antibodies against a *p*-TMI protein conjugate in a group of subjects in whom asthmatic reactions had been elicited by bronchial provocation testing with TDI at concentrations less than 0.02 parts/10⁶. In the first method, the *p*-TMI human serum albumin (HSA) conjugate was coupled to a solid phase—cellulose paper discs, whilst in the second method, *p*-TMI was reacted with HSA previously coupled to sepharose in an attempt to ensure maximum steric exposure of the tolyl groups.

The results in the patients with proven TDI-induced respiratory disease were compared with a group of workers exposed to TDI but without any respiratory disease as well as with groups of atopic and non-atopic individuals without any known contact with this chemical.

Materials and methods

Subjects

Sera were taken from five groups of subjects.

Group 1. Immediate reactors—workers exposed to TDI who had been shown on bronchial provocation challenge tests to develop immediate asthmatic reactions.

Group 2. Non-immediate reactors—workers exposed to TDI who had been shown on bronchial provocation challenge tests to develop non-immediate asthmatic reactions.

Group 3. Negative reactors—workers exposed to TDI but having no clinical symptoms.

Group 4. Non-exposed atopic—individuals not exposed to TDI who had been shown to be atopic on skin testing with common allergens.

Group 5. Non-exposed non-atopic—individuals not exposed to TDI who had been shown to be non-atopic on skin testing with common allergens.

Antigen preparation

The *p*-TMI was conjugated to the HSA by the method of Karol *et al.* (1978). 280 μ l of *p*-TMI (Eastman Chemical Company) was added to a rapidly stirred solution of 1% HSA, (Sigma Chemical Company Ltd) in 0.05 M borate buffer at pH 0.4, in an icebath. The conjugate was dialysed extensively, freeze dried, and spectroscopically examined at 242 nm (Fig. 1). A second conjugate of *p*-TMI and ϵ -amino caproic acid was prepared (Karol *et al.*, 1978) in order to calculate the number of mol of *p*-TMI per mol of HSA in the conjugate. The molar ratio was determined from the 242 nm absorbance

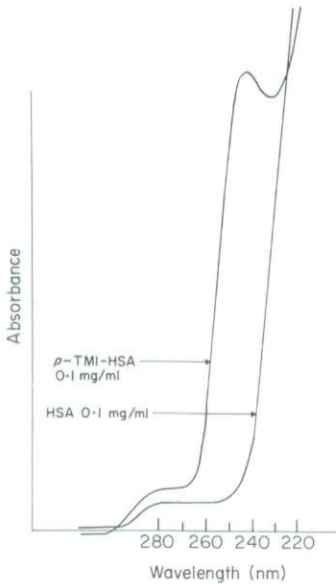


Fig. 1. Spectroscopic examination of HSA and *p*-TMI-HSA. Ultraviolet absorption spectra. HSA and *p*-TMI-HSA conjugates both at a concentration of 0.1 mg/ml in 0.05 M phosphate buffer, pH 7.0.

of the conjugate in excess of that due to HSA. It was calculated that there were 57 mol of *p*-TMI per mol of HSA.

Radioimmunoassay for specific IgE antibodies

In the first method the *p*-TMI-HSA conjugate was coupled to paper discs (Whatman No. 1 filter paper) activated by the method of Ceska & Lundkvist (1972). Eight ml of conjugate at a concentration of 10 mg/ml, in 0.1 M NaHCO₃ (pH 8.5) was added to 300 mg of activated paper discs and the mixture incubated at room temperature for 6 hr. The discs were washed three times in 0.1 M ethanolamine solution in 0.1 M NaHCO₃ solution (pH 8.5) and left overnight at 4°C. The discs were washed three times in Phadebas RAST assay buffer (Pharmacia Ltd) and incorporated in the RAST as described by Pharmacia (Pharmacia Diagnostics Ltd). The second method was based on that described by Cromwell *et al.* (1979). Fifty µl of *p*-TMI was added to 10 ml of activated sepharose 4B coupled to HSA in 10 ml of phosphate buffered saline (PBS) at pH 7.3 and mixed for 18 hr at 4°C. The suspension was then washed three times in PBS (pH 7.3) diluted 1 in 2 and used in the radioimmunoassay.

Total IgE

The Phadebas IgE PRIST kit (Pharmacia Ltd) was used to determine total IgE levels in all sera.

Statistical tests

A non-parametric test, the Mann-Whitney U test, was used to test for significant differences between the results for each of the groups.

Results

The results of the tests for specific IgE antibody against *p*-TMI are expressed as counts per minute (ct/min) and the measurement of total IgE antibody as units per ml (u ml^{-1}).

Figures 2 and 3 show the results of the test for specific IgE antibody against the *p*-TMI-HSA conjugate coupled to filter paper discs. In Fig. 2 the results are shown for

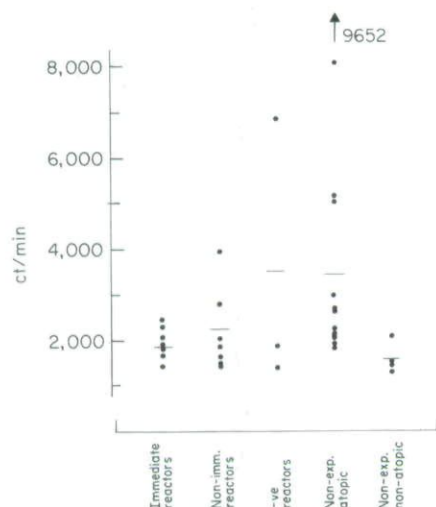


Fig. 2. Results of the radioimmunoassay using *p*-TMI-HSA conjugated to paper discs to test for specific IgE antibody in the sera of individuals in the five groups. Vertical axis: ct/min. Horizontal axis: Immediate reactors = immediate reactors exposed to TDI; non-imm. reactors = non-immmediate reactors exposed to TDI; —ve reactors = negative reactors exposed to TDI; Non-exp. atopic = atopic subjects not exposed to TDI; Non-exp. non-atopic = non-atopic subjects not exposed to TDI; — = mean ct/min.

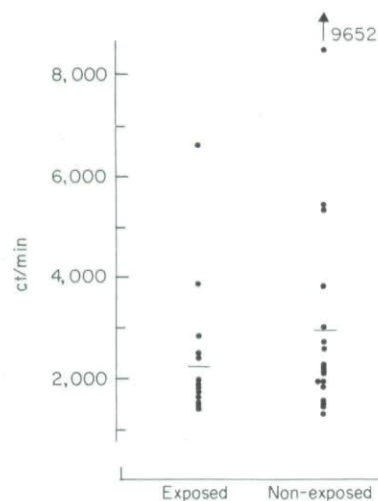


Fig. 3. The combined results of the radioimmunoassay using *p*-TMI-HSA conjugated to paper discs to test for specific IgE antibody in individuals exposed to TDI and those having no contact with this chemical. Vertical axis: ct/min. Horizontal axis: Exposed = subjects exposed to TDI; Non-exposed = subjects not exposed to TDI; — = mean ct/min.

the sera from individuals in each of the five groups. In Fig. 3 the groups have been combined into those individuals who were exposed to TDI and those who had never been in contact with the chemical.

Figures 4 and 5 show the results of the tests for specific IgE antibody against *p*-TMI reacted with sepharose coupled to HSA. In Fig. 4 the results are shown for the sera from individuals in each of the five groups. In Fig. 5 the groups have been combined into those individuals who were exposed to TDI and those who had never been in contact with the chemical. There was no difference in the results obtained by the two radioimmunoassay methods.

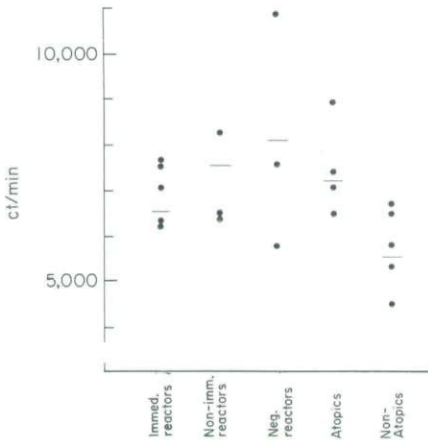


Fig. 4. Results of the radioimmunoassay using HSA-Sepharose beads reacted with *p*-TMI to test for specific IgE antibody in the sera of individuals in the five groups. Vertical axis: ct/min. Horizontal axis: Immediate reactors = immediate reactors exposed to TDI; Non-imm. reactors = non-immediate reactors exposed to TDI; Neg. reactors = negative reactors exposed to TDI; Non-exp. atopic = atopic subjects not exposed to TDI; Non-exp. non-atopic = non-atopic subjects not exposed to TDI; — = Mean ct/min.

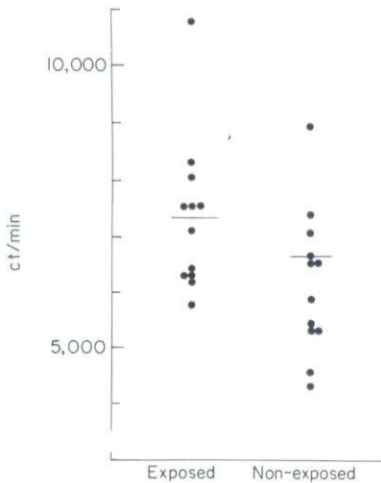


Fig. 5. The combined results of the radioimmunoassay using HSA-Sepharose beads reacted with *p*-TMI to test for specific IgE antibody in individuals exposed to TDI and those having no contact with this chemical. Vertical axis: ct/min. Horizontal axis: Exposed = subjects exposed to TDI; Non-exposed = subjects not exposed to TDI; — = mean ct/min.

In the radioimmunoassay using paper discs, the highest counts were obtained in the group of atopic subjects not exposed to TDI. Results for this group were found to be significantly higher than those for the group of non-atopic subjects not exposed to TDI ($P < 0.02$) and those for the group of immediate reactors exposed to TDI ($P < 0.05$). There was no significant difference between the results obtained for the group of immediate reactors compared to the group of non-immediate reactors. When the groups were combined, no significant difference was found between the results from the TDI-exposed subjects and subjects not exposed to TDI.

Table 1. A comparison of the ct/min in the two radioimmunoassays designed to test for the presence of specific IgE against *p*-TMI with the total levels of IgE (μml^{-1}) in the same sera.

Subjects	<i>p</i> -TMI-HSA Conjugate paper discs (ct/min)	HSA-Sepharose beads reacted with <i>p</i> -TMI (ct/min)	Total IgE (μml^{-1})
Immediate reactors	1698	6313	2
	1465	7068	3
	1727	6254	76
	2051	7519	150
	2469	7612	115
	1816	NA	42
	2067	6396	280
Non-immediate reactors	2781	8227	420
	1495	6457	70
	1480	NA	42
	1627	NA	84
	3816	8074	940
Negative reactors	2416	7571	190
	7141	10,895	580
	1861	5797	58
Atopic non-exposed	5679	NA	480
	2991	7079	250
	1808	6529	35
	5537	8954	> 1000
	2756	7389	370
	9652	NA	> 1000
	1961	NA	260
	2089	NA	460
	2601	NA	450
	2245	NA	490
	3951	NA	> 1000
	1917	NA	300
	2123	NA	330
Non-atopic non-exposed	1483	5854	12
	2074	6674	55
	1555	6532	15
	1297	5340	54
	1524	4550	2

NA = results not available.

Table 1 shows the ct/min in the two radioimmunoassays for all the sera tested. These results are compared with the total levels of IgE in the same sera. All the sera with higher levels of total IgE ($> 100 \text{ u ml}^{-1}$) had higher counts in the radioimmunoassay looking for specific IgE against *p*-TMI.

Discussion

We were unable to detect specific IgE antibodies to *p*-TMI-HSA in sera from workers who had asthma proven by bronchial provocation tests with TDI. High counts were obtained in both radioimmunoassays in some subjects with TDI-induced respiratory disease, but equally high counts were obtained with sera from atopic individuals who had never been exposed to this chemical. In our study the sera giving high counts in the radioimmunoassays with *p*-TMI-HSA also had high levels of total IgE. This suggests that these high counts simply reflect high levels of total IgE. This work does not confirm the study of Karol *et al* (1978a,b) in which specific IgE antibodies to *p*-TMI-HSA were found in three out of four TDI-exposed workers who had respiratory disease. There are a number of differences between the two studies which could account for the lack of agreement. Our study included a larger number of TDI-exposed workers, twelve subjects compared to the four investigated by Karol *et al* (1978a,b), all of whom had an asthmatic reaction elicited by bronchial provocation testing. Further, the total IgE levels in the sera of the subjects studied by Karol *et al* (1978a,b) were all below 100 u ml^{-1} . In our study 19 out of 33 subjects had levels of IgE greater than 100 u ml^{-1} . It was in the sera from this group of subjects that high counts in the radioimmunoassays were obtained. The *p*-TMI-HSA conjugate used in this study was prepared exactly as described by Karol *et al* (1978a,b). However, spectroscopic examination of the conjugate revealed a difference in the degree of substitution. The conjugate prepared by Karol and co-workers was calculated to contain 10 mol of *p*-TMI per mol of HSA. In our conjugate there were 57 mol of *p*-TMI per mol of HSA. We are unable to explain this difference.

Although we were unable to confirm the results of Karol and co-workers, it may still be possible that reagenic mechanisms are involved in the pathogenesis of TDI-induced respiratory disease. Radioimmunoassays designed to test for the presence of specific IgE against this chemical rely on the use of the correct antigen. At present there is insufficient information about the chemical nature of the adduct or adducts formed between inhaled TDI and the constituents of the respiratory tract and it is quite possible that such products could be the causal allergens. Alternatively, non-reagenic mechanisms may be involved. The disease may be the result of other types of allergic reaction or it may have a pharmacological basis. It has been shown, at least *in vitro* using the human lymphocyte model, that TDI has beta adrenoceptor blocking activity which could, in theory, contribute to the production of asthma (Davies *et al.*, 1977).

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