

Short communication

Detection of specific IgE in isocyanate and phthalic anhydride exposed workers: comparison of RAST RIA, Immuno CAP System FEIA, and Magic Lite SQ

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Sera from 94 workers occupationally exposed to isocyanate were tested by RAST RIA, Immuno CAP FEIA, and Magic Lite SQ (ML) against the allergens HDI, MDI, TDI, and phthalic anhydride. Twenty sera showed increased levels of diisocyanate-specific IgE antibodies, and five sera were phthalic anhydride positive. High total IgE titer was not correlated with positive specific IgE titer ($r = 0.47$), showing that nonspecific IgE binding was low. The results of ML and CAP correlated well ($r = 0.91$), but tended to be slightly higher than the results obtained with the RAST isotope test. CAP and RAST data also correlated significantly ($r = 0.90$), but the correlation between ML and RAST data was lower ($r = 0.82$). The results of the new *in vitro* tests, CAP and ML, showed good reproducibility (5% CV for CAP and 8% CV for ML). In summary, the CAP and ML methods were found to be appropriate for routine diagnosis of specific IgE antibodies against the allergens HDI, MDI, TDI, and phthalic anhydride.

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For many years, the radioallergosorbent test (RAST, Pharmacia Diagnostics AB, Freiburg, Germany) has been the standard method to monitor allergen-specific IgE antibodies in human serum. In this system, the allergens are covalently coupled to paper disks, and allergen-bound IgE antibodies are detected by ¹²⁵I-labeled anti-IgE antibodies. The specific allergen-antibody reactivity of unknown samples can be quantified by Phadebas RAST reference reagents, reported in Phadebas RAST units (PRU/ml), and classified in allergy classes (0-5).

Recently, two new methods to determine allergen-specific IgE antibodies have been introduced: the Immuno CAP System FEIA, a fully automated fluorometric system (Pharmacia Diagnostics, Freiburg, Germany), and the Magic Lite SQ specific IgE method, an immunochemoluminometric assay (Ciba Corning Diagnostics GmbH, Fernwald, Germany).

In this study, we evaluated these three immunoas-

says for detection of specific IgE antibodies against low-mol.-wt. chemicals. Low-mol.-wt. chemicals are thought to act as haptens and to become complete antigens after reaction with self-proteins (3, 5). Ninety-four serum samples from workers occupationally exposed to diisocyanates, and in some cases phthalic anhydride, in the isocyanate-processing industry were investigated, and the IgE antibody titer against the solid-phase allergens hexamethylene diisocyanate (HDI), methylene diphenyl diisocyanate (MDI), toluene diisocyanate (TDI), and phthalic anhydride were measured.

Material and methods

Workers studied

Serum samples were obtained from 94 workers occupationally exposed to common types of the isocyanates MDI, TDI, HDI, and, in some cases, to

phthalic anhydride. The exposed subjects originated from different plants. Sixty-three workers were engaged in polyurethane foam production. They were exposed mainly to MDI, to a lesser extent to TDI and HDI, and sporadically to phthalic anhydride. Ten workers were technicians in research and development laboratories and were exposed mainly to MDI, but also to TDI and occasionally phthalic anhydride. Twenty-one workers were outpatients, employed in car paint spraying, and production of motor-car accessories, condensers, and rigid and flexible foams. Some outpatients had been exposed exclusively to HDI; others to MDI, TDI, and phthalic anhydride. The study group comprised 85 men and nine women with ages ranging from 25 to 50 years. The mean duration of occupational exposure was 7.25 years (range 2–29 years).

The 21 IgE positives (17 men, four women; 13 smokers, five nonsmokers, three unknowns) showed work-related symptoms in the upper and/or lower respiratory tract or skin reactions. Of these, nine showed obstructive ventilation pattern, 10 nonspecific bronchial hyperreactivity, and two normal lung function.

Determination of allergen-specific IgE antibodies and total IgE

Phadebas RASTRIA. RAST was performed according to the manufacturer's instructions. The Phadebas RAST reference system 0.35–52.5 PRU/ml was used for quantification; 0.35 PRU/ml was defined as the cutoff for a positive/negative reaction (2).

CAP System FEIA. The CAP fluorescence enzyme-immunoassay is a fully automated system (Pharmacia Diagnostics AB, Freiburg, Germany). Standard or allergen CAPs were incubated for 30 min with 50 μ l of standard serum or sample. After automatic washing, 50 μ l of anti-IgE- β -galactosidase was added and incubated for 150 min. Unbound anti-IgE conjugate was washed away and 50 μ l 4-methylumbelliferyl- β -D-galactoside was added to develop fluorescence. After a 10-min incubation, 400 μ l of stop solution was added. The fluorescence signals of the eluates were read in the fluorocount 96. The system is calibrated against WHO standard for IgE to report results in international units (0.35–100 kU/l). The cutoff value for positive/negative is 0.35 kU/l.

Magic Lite SQ IgE specific. An amount of 250 μ l calibrator or standardized allergen (ALK Laboratories, Horsholm, Denmark) bound to paramagnetic particles (Ciba Corning, Fernwald, Germany) was incubated with 25 μ l reference serum or sample for 30 min. Unbound IgE was separated by magnetic

separation and decantation. After a wash step, 100 μ l acridinium ester-labeled anti-IgE was pipetted. The tubes were incubated for 30 min, followed by two wash cycles. Bound acridinium ester-labeled anti-IgE was measured by Magic Lite Analyzer II. Released photons were counted for 2 s and expressed as relative light units (RLU). The Magic Lite Analyzer II calculates the reference curve on the basis of a master curve (10-point calibration), together with a low and high calibrator which fixes the relevant curve end points. Results were expressed in standard units (SU/ml). The cutoff value was 1.42 SU/ml.

Determination of total IgE

Total serum IgE was measured by Magic Lite Total IgE extended range (Ciba Corning, Germany).

Statistical analysis

All data were analyzed after log transformation. We used the SPSS/PC⁺ program software to calculate the linear correlation coefficient among the results of RAST RIA, CAP FEIA, and Magic Lite SQ.

Results

Of the 94 sera analyzed, 20 showed increased levels of diisocyanate-specific IgE antibodies by all the methods used. Table 1 shows the individual antibody levels reported in allergy classes. Of the 20 probes, 19 were found positive for HDI, 18 for TDI, and 17 for MDI. For the HDI allergen, 17 were identified as positive by ML, 16 by CAP, and 15 by RAST; for the MDI allergen, 16 were identified as positive by CAP, 13 by ML, and 11 by RAST; for the TDI allergen, 16 were identified as positive by ML, 15 by RAST, and 15 by CAP.

Phthalic anhydride specific IgE antibodies were identified in five of the 94 samples by ML, in two by CAP, and in one by RAST (Table 1).

A highly significant correlation was found between ML and CAP data when the calibrated units (PRU, kU, SU) were compared on a point-by-point basis, especially for the HDI and MDI allergens (r HDI, MDI = 0.96; r TDI = 0.92). Good correlations were also found between CAP and RAST (r HDI = 0.95; r MDI = 0.89; r TDI = 0.88). The correlation between ML and RAST (r HDI = 0.89; r MDI = 0.84), especially for the TDI allergen (r = 0.77), was less satisfying. By calculation of the test results for the three diisocyanate allergens together, the best correlation was found for ML and CAP (r = 0.91) and one nearly as good for CAP and RAST (r = 0.90).

Since isocyanate solid-phase allergens were prepared with HSA conjugates, our investigation in-

Table 1. Isocyanate exposure and positive test results in 34-person study group using the three solid-phase immunoassays RAST, CAP, FEIA, and Magic Lite SQ. Allergy classes are listed for the isocyanate allergens HDI, MDI, and TDI and for phthalic anhydride (PA).

Isocyanate exposure			Sample No.	HDI			MDI			TDI			PA		
				RAST	CAP	ML	RAST	CAP	ML	RAST	CAP	ML	RAST	CAP	ML
*HDI	MDI	TDI	1	1	0	0	2	0	0	0	0	0	0	0	0
HDI			2	2	2	2	0	0	0	1	1	1	0	0	0
	MDI	TDI	3	1	2	2	0	2	0	1	2	1	2	3	3
HDI			4	0	1	2	0	0	0	0	0	0	0	0	0
	MDI	TDI	5	5	5	5	5	6	5	5	5	5	0	1	3
	MDI		6	0	1	2	0	1	0	0	1	2	0	0	1
	MDI		7	0	0	1	1	2	1	0	1	1	0	0	0
HDI			8	2	2	3	0	1	0	2	2	1	0	0	0
HDI			9	2	3	3	0	1	1	2	2	2	0	0	2
*HDI	MDI	TDI	10	0	1	2	2	2	2	1	1	2	0	0	0
*HDI	MDI	TDI	11	1	1	2	2	2	2	1	1	1	0	0	0
*HDI	MDI	TDI	12	1	1	1	0	2	2	1	0	1	0	0	0
*HDI	MDI	TDI	13	2	2	2	3	3	3	2	2	1	0	0	0
*HDI	MDI	TDI	14	3	3	3	4	4	3	3	3	3	0	0	0
*HDI	MDI	TDI	15	2	2	3	3	3	3	2	3	2	0	0	0
	MDI		16	0	0	0	0	0	0	1	0	0	0	0	0
	MDI		17	2	2	2	3	3	2	2	2	2	0	0	0
	MDI		18	1	0	0	1	1	1	1	0	0	0	0	0
*HDI	MDI	TDI	19	4	3	4	5	4	4	3	3	2	0	0	0
HDI			20	1	2	2	0	2	1	0	2	1	0	0	0
*HDI	MDI	TDI	21	0	0	0	0	0	0	0	0	0	0	0	1

* Sporadic.

cluded the assessment of IgE antibody binding to HSA. All 21 samples with specific IgE antibodies to diisocyanates and/or phthalic anhydride were checked for IgE antibodies against HSA in the ML assay. Only sample no. 12 showed a slightly elevated value of 1.5 SU/ml. The other 20 samples with specific IgE antibodies to diisocyanates and/or phthalic anhydride showed no cross-reacting IgE antibodies to the carrier molecule HSA.

Unspecific IgE binding was further examined by comparison of total serum IgE levels with specific IgE levels. Although some subjects with isocyanate specific IgE had a high total IgE level, high total IgE titer was not correlated with positive specific IgE titer ($r = 0.47$).

Discussion

The aim of this study was to examine the two new *in vitro* methods, CAP and ML, to determine isocyanate and phthalic anhydride specific IgE antibodies. Since the isocyanate carrier protein conjugates are difficult to prepare (1, 9) and important for the IgE binding to solid-phase allergens (9), we compared the results of the two new methods with that of the well-established RAST RIA. We found that the new methods ML and CAP can replace RAST RIA (Table 1). In general, ML and CAP yield higher results which correlate well ($r = 0.91$). Other groups have investigated high-mol.-wt. aeroallergens by

CAP and RAST and reported similar coefficients of 0.82–0.91 and 0.864–0.95, respectively (4, 8).

With regard to isocyanate exposure (Table 1), most positive samples (except nos. 4 and 16) showed IgE antibodies against two or three diisocyanates. The finding of cross-reactivity is not surprising, as high cross-reactivity of isocyanate-specific antibodies is well known (1, 10) and varies among subjects (9).

In our study group, all IgE-positive subjects showed work-related symptoms. This finding does not fully agree with results from other groups. Butcher et al. (5) and Butcher & Salvaggio (6) mentioned that care must be taken when RAST results are evaluated, as illustrated by the finding that some asymptomatic isocyanate-exposed workers demonstrated tolyl-specific IgE antibodies. In contrast, Cartier et al. (7) found that approximately 30% in their study group showed positive isocyanate inhalation challenge (HDI, MDI) without increased IgE and/or IgG levels. They demonstrated an association between positive specific inhalation challenge and the presence of specific IgG antibodies, but not of IgE.

Despite these findings, we consider that the isocyanate-specific IgE titer may be helpful and give the clinician a further criterion for the diagnosis of occupational respiratory diseases. A positive test result may suggest that the workers' symptoms are mediated by specific IgE antibodies and can confirm the diagnosis. The advantage of the new methods

ML and CAP is the avoidance of handling the unstable and hazardous isotopic reagents which are necessary for RAST RIA.

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