

Clinical and immunologic evaluation of workers exposed to glutaraldehyde

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We describe immunologic responses in subjects exposed to glutaraldehyde (GA) who were diagnosed as having occupational asthma, or who described work-related respiratory symptoms. A series of GA-modified proteins was characterized, and used to analyse sera from 20 GA-exposed workers and 21 unexposed workers for IgE antibodies. Inhibition studies were used to determine the specificity of binding. The reaction of GA with albumin in different molar ratios produced a range of modified proteins, which were used to measure specific IgE antibodies. A significant difference between exposed and unexposed subjects with serum IgE less than 150 kU/l could be detected for GA-specific IgE antibodies ($P=0.026$), and 31% of exposed workers with occupational asthma had antibody levels greater than the unexposed population (mean +2.5 SD). False-positive results were obtained with serum from unexposed workers who had total IgE levels greater than 150 kU/l, but this binding was not inhibited by GA-modified proteins. We report the first evidence of immunologic sensitization in some workers exposed to GA. However, GA may behave like many other low-molecular-weight chemicals in that specific antibodies can be detected in only a small percentage of exposed workers who report work-related respiratory symptoms.

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The inhalation of airborne low-molecular-weight chemicals can cause respiratory sensitization in susceptible patients. In this paper, we present the first report of immunologic responses to the low-molecular-weight chemical glutaraldehyde (GA) ($\text{OHCC}_3\text{H}_6\text{CHO}$). GA is used as a disinfectant or a sterilizing agent, and is often a constituent of radiographic developing solutions. The biocidal properties of the compound are also utilized by the offshore oil and gas industries, where it is added to well-injection sea water to keep contaminating microbes to a level whereby their waste products do not corrode the drilling machinery. It has been reported to cause various health problems including dermatitis, upper respiratory tract irritation, and occupational asthma (1, 2). Most cases have been reported among health-care professionals, particularly radiographers and theatre nurses with responsibility for instrument sterilization. Some instrument sterilization systems are partially enclosed, and use local exhaust ventilation to reduce exposure. However, the operation is often

performed in large open trays containing a 2% GA solution. The UK Health and Safety Executive has set a short-term exposure limit of 0.7 m gm^{-3} (15-min reference period) for GA (3). Routine exposure of workers to airborne concentrations of the compound has been shown to fall within this limit (1), and a recent report has described no indications of GA-induced skin or respiratory sensitization in workers exposed to GA at or below this level (4). However, it has been observed that the limit can be exceeded when specific tasks are performed (5).

Most reports in the literature pertaining to the health effects of GA exposure are anecdotal, although some groups do present clinical evidence of sensitization. Peak-flow measurements have been used to show a significant reduction in peak expiratory flow rate (PEFR) when the head nurse of an endoscopy unit used GA for sterilization (6). This reduction was not observed when a substitute disinfectant was used. A more recent study used workplace challenge testing of a respiratory

technologist and showed a similar decrease in FEV₁ and PEFR after working with 3.6% GA (7). In this case, lung function had not returned to normal 24 h after challenge, a finding which indicated the occurrence of a late asthmatic response. Bronchial provocation testing of a radiographer with 11% GA solution provoked a late asthmatic response at 3 h with a reduction by over 50% in her pretest histamine PC₂₀ (8). More recent work has shown that 7/7 subjects who had significant work-associated reductions in workplace serial peak-flow measurements also had a significant reduction in PEFR with a characteristic late-phase reaction after bronchial provocation testing with 2% GA (9).

However, while there is clinical evidence suggesting sensitization by this simple compound, there is very little immunologic evidence to support these observations. The cross-linking of proteins by GA is well documented (10, 11), but the immunologic consequences of this action are not understood.

To address this question, we have studied 20 patients exposed to GA, 13 of whom had GA-induced occupational asthma as diagnosed by a respiratory physician. IgE antibodies were measured, and compared to the levels seen in a group of nonexposed subjects.

Material and methods

Subjects

Twenty subjects exposed to GA were studied. Thirteen of them had occupational asthma diagnosed by a respiratory physician (P. S. Burge). All patients had been referred to the Birmingham Chest Clinic for further investigation. Seven of the patients studied had been challenge tested at the clinic (9).

Serum samples

Venous blood (10–20 ml) was taken from each subject for specific antibody measurement. Serum was separated and stored at –20°C until required.

Production and characterization of GA-modified proteins

GA was used to modify both bovine serum albumin (BSA) and human serum albumin (HSA) by a modification of the technique used to synthesize hapten-protein conjugates (12). Briefly, different molar ratios of 20% GA (Sigma, UK) and 2% w/v protein solutions (molar ratio range from 2.9 to 8732.5) in 20 ml phosphate-buffered saline (PBS)

(pH 7.4) were tumble mixed overnight at room temperature. The resultant solutions were dialysed against PBS (pH 7.4), and their protein content was estimated by the bicinchoninic acid method (13). Protein modification was confirmed by spectrophotometry, since GA-modified proteins contain quaternary pyridinium groups which have a $\lambda_{\max}$ chain of about 265 nm (10). Therefore, the ratio of absorbance at 265 nm to absorbance at 280 nm was used as a qualitative measure of protein modification by GA, after correction for protein concentration.

Preparation of allergen discs

The method of Ceska et al. (14) was used to activate paper discs (Schleicher and Schuell, Inc., Keene, NH, USA; no. 589) with cyanogen bromide (Sigma, UK). Activated discs were tumble mixed overnight with GA-modified HSA (GA-mHSA; molar ratio of 2183 : 1) or 2% HSA in 0.1 mol/l of sodium bicarbonate at room temperature at a ratio of 100 : 1 (w/w). The discs were washed four times with PBS (pH 7.4), and stored in RAST buffer solution at –20°C until required.

Radioallergosorbent test (RAST)

Serum (200 µl of 1 : 4 dilution in PBS, pH 7.4) was incubated overnight at room temperature with GA-modified HSA and HSA discs. Discs were washed four times with 1.2 ml of 0.9% saline containing washing solution additive (Pharmacia, Uppsala, Sweden), and then 100 µl of ¹²⁵I-labelled rabbit antihuman IgE (Pharmacia, Uppsala, Sweden) was added. After an additional overnight incubation at room temperature, the discs were washed four times, and bound ¹²⁵I was measured in a gamma counter (Canberra-Packard). All the assays were performed in duplicate. The results were expressed as RAST per cent binding, which was defined as the percentage of total added counts per minute bound to the GA-mHSA disc incubated with the test serum, minus the percentage of added counts per minute bound to the HSA-disc incubated with the test serum.

Total serum IgE

Total serum IgE levels were measured with a RIACT kit (Pharmacia, Uppsala, Sweden) according to the manufacturer's instructions.

RAST inhibition

For RAST inhibition, 50-µl aliquots of serum diluted in an equal volume of PBS were mixed with

100 µl of PBS or 100 µl of inhibitor diluted in PBS, and allowed to preincubate for 30 min. The inhibitors used were GA-mHSA, GA-modified BSA, albumin alone, and an irrelevant conjugate of trimellitic anhydride coupled to HSA. All were used at concentrations between 16 µg/ml and 2000 µg/ml. A GA-mHSA disc was then added to each tube, and the tubes were incubated overnight at room temperature. Each inhibitor concentration was tested in duplicate. The value for 100% binding was determined by incubating discs with 100 µl of the same dilution of test serum plus 100 µl of PBS. IgE bound to the discs was measured as described for specific IgE. The results were expressed as per cent inhibition.

Statistical analysis

The Mann-Whitney U nonparametric test was used to compare test and control populations, and correlation coefficients were calculated with the C-Stat statistical package.

Results

Subject characteristics

There were 18 female and two male subjects in the study population with mean age 46.7 (± 11.4 years).

All were health-care professionals (nurses, dentists, laboratory technicians) who were routinely exposed to GA in their normal work. However, most had been removed from exposure for at least 6 months at the time the blood samples for this study were taken. Twenty-one archived sera from unexposed subjects were randomly selected as controls.

History and clinical assessment

Of the 20 subjects investigated who were exposed to GA during the course of their work, 13 had clinically diagnosed occupational asthma; the remainder reported respiratory symptoms when working with GA. Seven of the eight subjects who were challenge tested gave a positive reaction when challenge tested with GA. These data are summarized in Table 1.

Production and characterization of GA-modified proteins

Fifteen different ratios of 20% GA and 2% albumin were used to produce GA-modified proteins. Scanning spectrophotometry of the GA-modified proteins between 200 and 300 nm showed an increase in absorbance at 265 nm with increasing GA concentration, demonstrating the cross-linking effect on the protein (Fig. 1); GA did not absorb

Table 1. Demographic, clinical, IgE antibody, and exposure data of GA-exposed study population. Subjects 3 and 10 provided multiple samples

Subject	Occupation	Age (years)	Sex	Diagnosis	Challenge test result	Months since last exposure	RAST % binding	Total IgE (kU/l)
1	Secretary in radiography department	40	F	OA	Positive	0	0.37	18.9
2	Endoscopy nurse	51	F	OA	Positive	6	0.39	37.7
3	Endoscopy nurse	48	F	OA	Positive	0	1.09	120
3	—	—	—	—	—	6	0.67	72.4
3	—	—	—	—	—	9 ^a	1.21	120
4	X-ray technician	53	F	OA	Positive	0	0.28	NT
5	Nurse	48	F	OA	Positive	0	0.36	3.7
6	ENT nurse	29	F	OA	Positive	0	0.27	73
7	Day-care unit manager	33	F	OA	Positive	12	1.44	2
8	Endoscopy nurse	58	F	OA	NT	12	0.71	77.1
9	Endoscopy nurse	57	F	OA	NT	12	0.41	17.4
10	X-ray technician	61	F	OA	NT	0	0.58	36.4
10	—	—	—	—	—	6	0.43	54.7
11	Dentist	52	M	OA	NT	0	1.61	376
12	Theatre nurse	58	F	OA	NT	0	2.67	480
13	Retired nurse	63	F	OA	NT	0	0.7	4.4
14	Radiographer	35	M	RS	Negative	18	0.57	14
15	Endoscopy nurse	50	F	RS	NT	6	0.31	21.8
16	Histology technician	56	F	RS	NT	6	0.85	139
17	Radiographer	31	F	RS	NT	36	0.72	42
18	Mortuary technician	22	F	RS	NT	0	1.08	7.9
19	Theatre nurse	49	F	RS	NT	2	1.22	114.4
20	Radiographer	40	F	RS	NT	0	0.65	13.7

^a Sample taken after challenge test; NT: not tested; OA: occupational asthma to GA; RS: respiratory symptoms when working with GA.

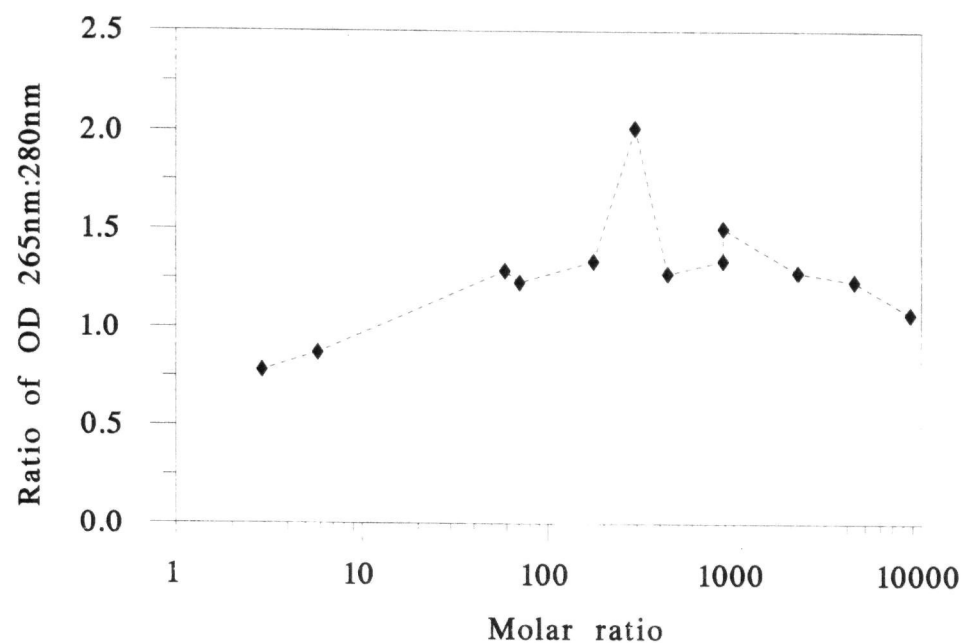


Fig. 1. Changes in ratio of optical density (OD) at 265–280 nm of GA-modified albumin when different molar ratios of GA to albumin were used in its preparation.

at either 265 or 280 nm. In general, the GA-modified proteins produced from higher GA ratios showed higher absorbance at 265 nm than at 280 nm, with a peak for GA-modified proteins with ratios greater than 70 : 1, reflecting the maximum number of available binding sites on the albumin molecule. HSA has 58 lysine residues which are prime candidates for both haptenization and cross-linking, closely reflecting this data.

RAST analysis and total IgE determination

HSA modified by GA (GA-mHSA) at a GA : protein molar ratio of 2183 : 1 gave the best results in RAST analysis, since the others showed a lack of sensitivity or increased nonspecific binding making them unsuitable for use in the assay (data not shown). All serum samples were subsequently tested for specific IgE with discs coated with this GA-mHSA.

The results obtained from RAST analysis were expressed as per cent binding, after the per cent binding to the HSA disc had been subtracted. Net RAST per cent binding of more than 0.88% was taken to indicate the presence of specific IgE in sera with total IgE equal to or less than 150 kU/l (Table 1). This value was the mean per cent binding of 21 sera from subjects not exposed to GA plus 2.5 times the standard deviation of this mean (99% confidence interval). Of the 13 subjects with occupational asthma, two had total IgE values greater than 150 kU/l, and 13 had values less than 150 kU/l. Of the exposed population with clinically diagnosed occupational asthma and total IgE less than 150 kU/l, 2/11 showed a positive RAST result as defined by the criteria above, whereas the unexposed subjects with serum IgE less than 150 kU/l were all

negative for specific IgE. A comparison of RAST per cent binding from both the exposed and non-exposed populations with total IgE less than 150 kU/l showed a significant difference in specific IgE levels between the two populations ($P=0.026$, Mann-Whitney U test) (Fig. 2).

Unexposed control sera with total IgE greater than 150 kU/l were found to give false-positive

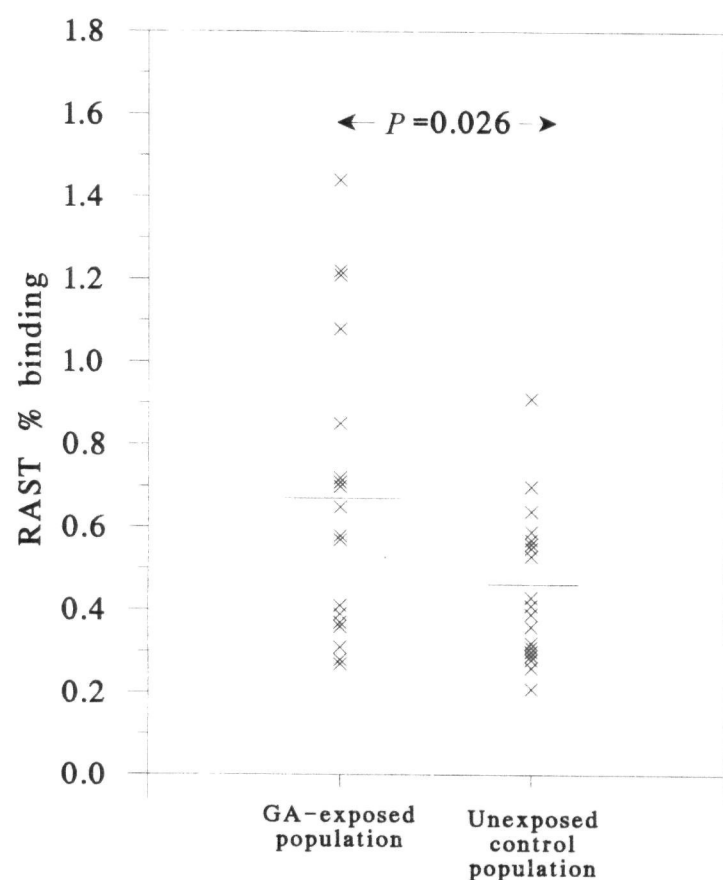


Fig. 2. RAST per cent binding of serum samples with total IgE <150 kU/l to GA-mHSA-coupled discs from population exposed to GA and unexposed control population comprising random archived sera. Significance was determined by Mann-Whitney U test.

results; therefore, correlation coefficients were calculated between RAST per cent binding to GA-mHSA and total serum IgE levels for all samples tested with IgE greater than, less than, or equal to 150 kU/l. There was no correlation between total IgE level and RAST per cent binding to GA-mHSA when total serum IgE levels were below 150 kU/l ($r=0.37$), but for samples with total serum IgE greater than 150 kU/l, the correlation coefficient increased ($r=0.66$).

RAST inhibition

RAST inhibition was used to assess the specificity of the binding seen with subject 3, who showed the most marked clinical response when challenge tested with GA (8). Fig. 3 shows the inhibition of binding to GA-mHSA discs (ratio 2183:1) with various inhibitors. Both GA-mHSA and GA-modified BSA could inhibit the binding of this serum to GA-mHSA allergen discs at concentrations greater than 400 $\mu\text{g/ml}$. No significant inhibition was seen with either HSA alone or an irrelevant albumin conjugate of trimellitic anhydride. No significant inhibition of binding to GA-mHSA discs (ratio 2183:1) was seen when either a serum from the unexposed population with a total IgE level of 116 kU/l (RAST per cent binding=0.57), or a serum with a total IgE level of 395 kU/l (RAST per cent binding=2.48) was preincubated with

GA-mHSA (ratio 2183:1) (Fig. 4). Therefore, antibodies from subject 3 were shown specifically to recognize GA-modified albumin. Serum samples were limited, so inhibition studies could not be performed on other subjects.

Discussion

Although we were able to detect a significant increase in IgE antibodies to GA-modified proteins in some subjects exposed to GA, the assay we describe was able to detect specific IgE to GA in only 2/11 (18.2%) subjects with total serum IgE less than 150 kU/l who had been clinically diagnosed as having occupational asthma. The two positive subjects identified by RAST also had a positive GA bronchial provocation test. While the RAST per cent binding to GA-mHSA discs was not high, it was significantly higher than the results from an unexposed population with serum IgE less than 150 kU/l ($P=0.026$, Mann-Whitney U test). A complicating factor was the length of time between the last exposure and the date that the sample was taken, since, in many cases, at least 6 months had elapsed since GA had been used. Obviously, this could lead to a reduction in specific antibody levels, producing a negative RAST result. Venables et al. (15) have demonstrated a drop in specific IgE in workers exposed to tetrachlorophthalic anhydride (TCPA) 6–8 months after their last exposure. At

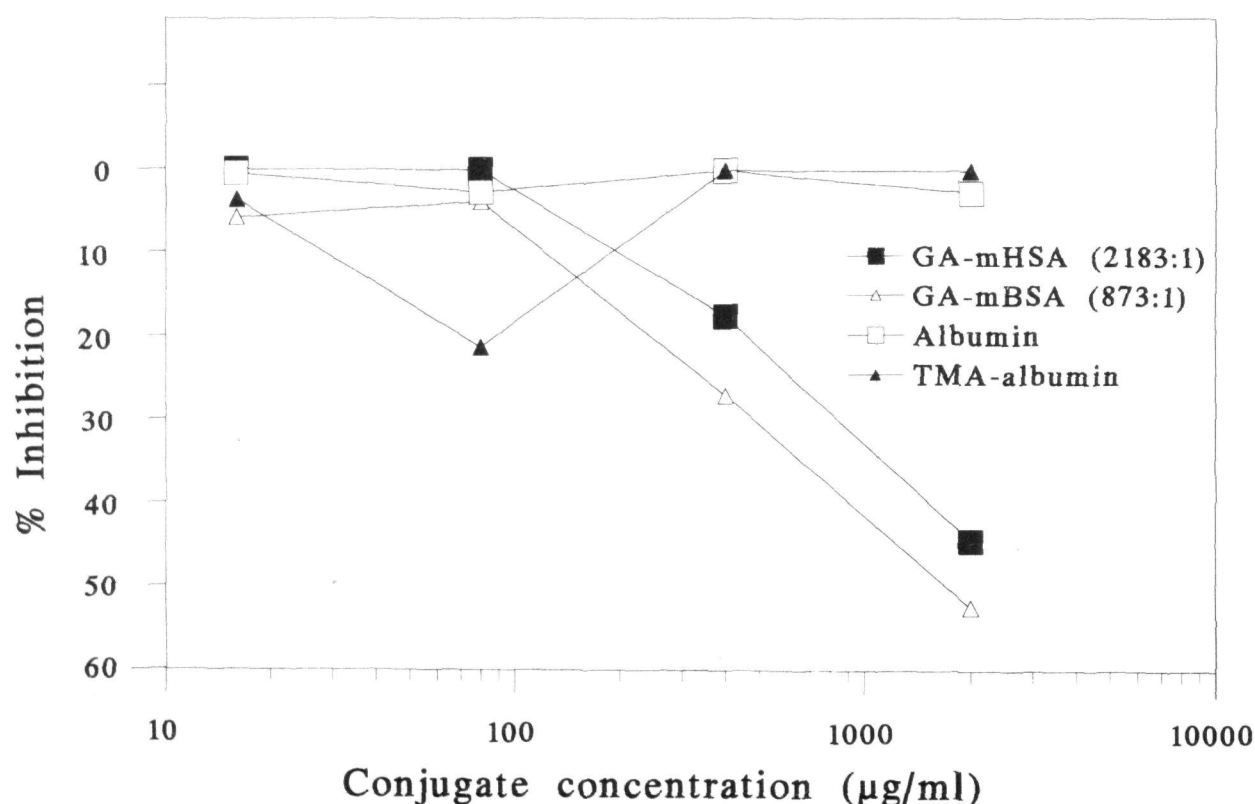


Fig. 3. Inhibition of binding of serum from subject 3 to GA-mHSA discs by preincubation with range of inhibitors. GA-mHSA = GA-modified HSA; GA-mBSA = GA-modified bovine serum albumin; albumin = HSA alone; TMA-HSA = trimellitic anhydride conjugated to HSA (irrelevant chemical-protein construct).

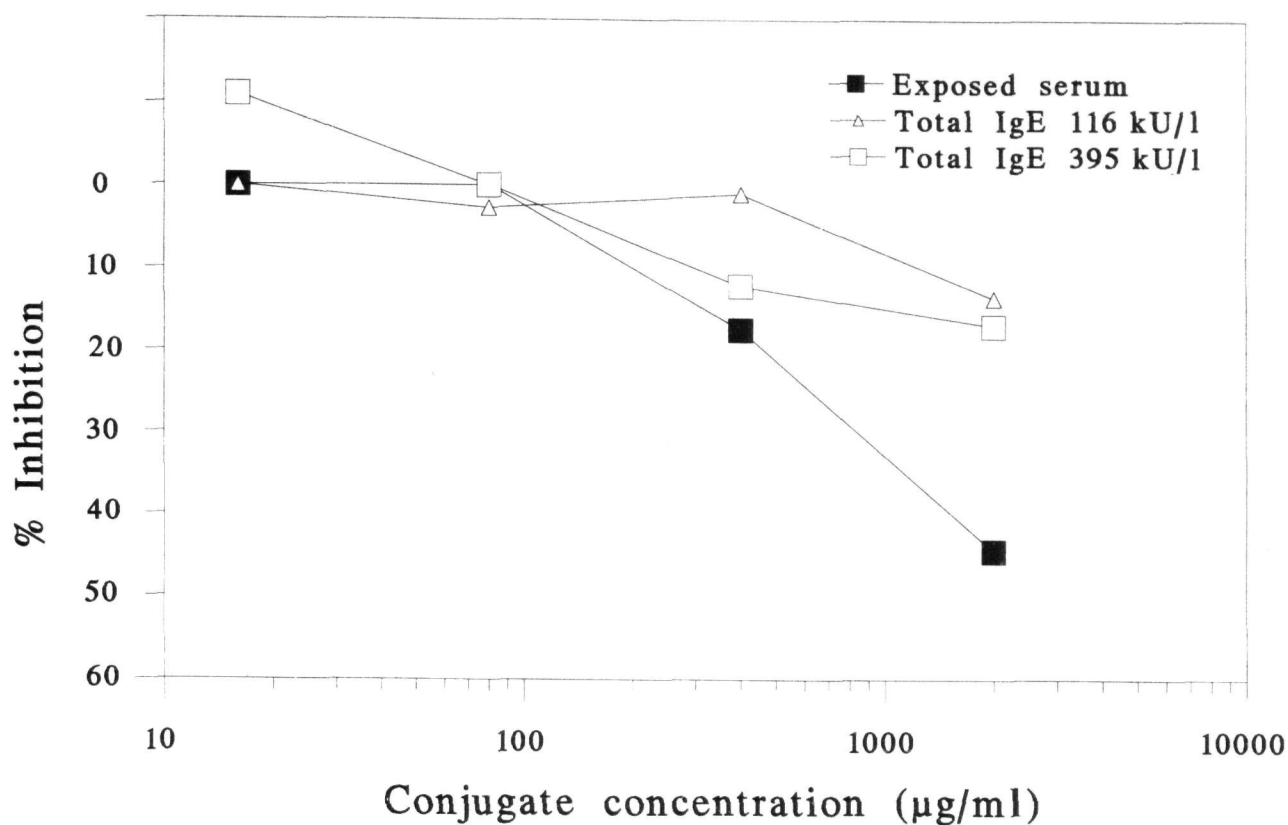


Fig. 4. Inhibition of binding of different sera to discs coated with GA-mHSA by preincubation with identical GA-mHSA conjugate.

this time, the workers had a TCPA inhalation challenge, and specific antibody levels were seen to rise when tested 2 months later.

For all samples analysed (20 exposed, 21 unexposed), there was some correlation ($r=0.66$) between total serum IgE and RAST per cent binding to GA-modified proteins when serum IgE levels were greater than 150 kU/l; this correlation was not seen when serum IgE was less than 150 kU/l ($r=0.37$). Therefore, the test was considered to be valid for samples with total IgE below this value. Further validation was provided by the RAST inhibition studies, since the binding to GA-mHSA discs of unexposed sera with total IgE of 116 and 395 kU/l was not inhibited by any of the materials tested, whereas serum from a subject with a positive bronchial provocation test was inhibited by GA-modified proteins. There was insufficient sample available to perform RAST inhibition on the two exposed subjects with total IgE greater than 150 kU/l, and we were unable to confirm the specificity of antibody binding.

Therefore, GA seems to belong to a growing group of low-molecular-weight chemicals for which there is a poor correlation between specific IgE antibody and clinical symptoms of occupational asthma. For example, the presence of isocyanate IgE has been demonstrated in only approximately 10–20% of symptomatic subjects, and has also been found in workers with a negative inhalation challenge (16). Similarly, there have been few reports of specific antibodies to formaldehyde despite the large volume of challenge data (17). While this may

be due to absence from the assay system of the new antigenic determinants produced by the action of such chemicals on lung proteins, the significance of serum IgE in the pathogenesis of the condition is not clear, and other immune and nonimmune mechanisms may play a role in the development of the condition. The crucial role played by the cells of the immune system in the pathogenesis of nonoccupational intrinsic asthma is now well documented (18), and there is growing evidence of T-cell activation in extrinsic (allergic) asthma (19). We are currently using *in vitro* techniques to investigate these responses, and to evaluate the importance of other mechanisms in the induction of occupational asthma.

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