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Specific IgE to colophony?

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Background Colophony (rosin) is a natural product obtained from coniferous trees. It is used in a diverse range of products such as adhesives, ink, paints and soldering fluxes. Some workers exposed to colophony during soldering can develop occupational asthma; at present, no specific IgE test is available to assess sensitization to colophony.

Methods Serum samples were obtained from exposed symptomatic individuals ($n = 7$), some with a likely diagnosis of occupational asthma, exposed asymptomatic individuals ($n = 10$) and unexposed individuals ($n = 11$). Serum was tested for specific IgE antibodies against a protein extract produced following *in vitro* challenge of mono-mac-6 cells with colophony extract.

Results Serum from exposed symptomatic individuals showed increased binding of specific IgE antibodies to a range of colophony–cell protein conjugates [29% (2/7) of samples tested when cut-off >0.1 or 86% (6/7) of samples tested when cut-off $>0\%$] compared with both the exposed asymptomatic [0% when cut-off >0.1, or 20% when cut-off $>0\%$ (2/10)] and the non-exposed control populations [0% when cut-off >0.1, or 27% when cut-off $>0\%$ (3/11)].

Conclusions This novel approach for the production of conjugates to assess sensitization to colophony was able to detect specific IgE in colophony-exposed workers with a likely diagnosis of occupational asthma.

Key words Colophony; rosin.

Introduction

Colophony (rosin) is a natural product obtained from coniferous trees. Due to its stickiness, emulsifying and insulating properties, the resin is a common ingredient in a diverse range of products such as adhesives, ink, paints and soldering fluxes. Unfortunately, it causes allergic skin and respiratory reactions. Occupational asthma occurs primarily in those employed soldering conductive connections in the electrical and electronics industries. The potential of colophony fumes to induce asthma has been recognized since the 1970s. The UK Surveillance of Work-related Occupational Disease (SWORD) scheme attributed some 5% of all new cases of occupational asthma to this cause in 1994 [1] although some cross-sectional studies have not been able to identify an excess of respiratory problems in solderers [2,3], others have shown an increased incidence of occupational asthma in exposed workers [4], in one study being as high in bystanders as in solderers themselves [5]. Exposure to soldering fumes was the most important risk factor for occupational asthma in one case–control study [6]. Occupational asthma in solderers continues to be reported and investigated by occupational health practitioners.

While the medical and work histories and responses to provocation tests of solderers with occupational asthma suggests an immunological basis for their disease, no direct *in vitro* or *in vivo* evidence for this has been reported [6–8]. Colophony fume is irritating both to the respiratory tract and the eyes and the true nature of respiratory symptoms can be difficult to determine. The demonstration of antibody responses in those reporting respiratory symptoms would be of great benefit in providing an objective marker between exposure and response. This would have use in both routine health surveillance and in epidemiological work establishing the relationship between symptoms and exposure to colophony.

There is uncertainty as to whether the resin acids in colophony, or thermal breakdown products from them, are the main cause of occupational asthma. Most inhalable exposure to colophony comes from heating, resulting in a mixture of resin acids and decomposition products. Differential challenge tests with colophony adducts suggests that both esterifying the acid radical and conjugating the double bonds both reduce

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Table 1. Clinical data from exposed symptomatic population

ID	Age	Job	Interval from first exposure to first symptom	FEV_1 % pred	FVC % pred	FEV_1/FVC	Total IgE	Atopic	Smoking	PD20 (methacholine)		Diagnosis/history
										Exposed	Not exposed	
1	48	Solderer	2 years	117	128	78	7	No	Never	NI	4800	Equivocal PEFs, history, possible occ asthma
2	28	Solderer	Has had asthma since childhood	106	110	85	NI	Yes	Ex	NI	3330	Definite asthma, possible occ asthma (inadequate/equivocal PEFs)
3	35	Solderer	4 months	84	89	81	76	Yes	Never	94	216	Very positive PEFs, positive challenge to non-colophony solder
4	45	Solderer	9 years (asthma), 2 years (rhinitis)	73	91	70	35	No	Never	1950	3272	One clear positive PEF. Positive challenge to non-colophony solder (asthma) and colophony solder (rhinitis)
5	28	Solderer	29 months	75	77	81	77	Yes	Never	713	1425	Strongly positive history, positive PEFs
6	52	Solderer/de-solderer	3 years	91	108	72	379	Yes	Current	NI	3.25 histamine	Positive challenge to colophony (immediate reaction). Good history, positive PEFs
7	56	Solderer of heated windscreen terminals	7 months	66	89	60	366	NI	Current	NI	138	Intermittently worse at work on PEFs, but improvement on leaving work Suggestive history, but not diagnostic. Possible occ asthma

NI = no information.

the asthmatic response to heated colophony [9]. Exposure to the particulates from ground unheated colophony has reproduced asthma in one worker [10], showing that decomposition products are not always required.

Previous work by our group has shown that low molecular weight chemicals including colophony components [11] and isocyanates [12] can induce oxidative burst in immune cells. We proposed that this might be a mechanism by which some low molecular weight chemicals can induce haptenization with body proteins.

In this study, we have used this observation to develop a technique to produce colophony–protein conjugates *in vitro*. Conjugates produced using this novel approach were tested against serum from exposed symptomatic, asymptomatic exposed and non-exposed individuals to see whether this approach has the potential to be utilized for the detection of specific IgE to colophony.

Methods

Symptomatic individuals ($n = 7$) from a range of different workplaces were recruited to the study population from a specialist centre for occupational respiratory disease. All individuals in the symptomatic group were exposed to colophony fume. A full medical and work history was obtained from each individual, and lung function was assessed (per cent predicted FEV_1 and FVC). A combination of bronchial hyper-reactivity and peak expiratory flow monitoring (using the OASYS system [13]) was also performed in order to confirm a diagnosis of occupational asthma in some of the subjects. Three workers had specific challenge testing to heated colophony in soldering flux.

A population of asymptomatic individuals exposed to colophony ($n = 10$) was recruited from a motor vehicle component manufacturer in the same geographic area as the symptomatic population. In addition, a population of non-exposed, asymptomatic volunteers ($n = 11$) was recruited from a population of office-based staff.

All study volunteers were asked to provide a 10 ml clotted blood sample for radioallergosorbent test (RAST) analysis.

Research Ethics Committee approval for the study to proceed was obtained from the Birmingham Heartlands and Solihull NHS Trust Research and Ethics Committee, and all volunteers provided written informed consent to participate in the study.

Production details of the colophony–cell protein conjugates and allergen disks for RAST analysis are given in full in the electronic journal.

Each individual provided a clotted blood sample for RAST analysis. The serum was separated from the sample and stored at −20°C until analysis. The sera were tested

for specific IgE antibodies to the colophony–cell protein conjugate discs, and the cell protein disc. Serum (200 μl of 1:4 dilution in PBS, pH 7.4) was incubated overnight at room temperature with the protein-coupled discs. The discs were washed four times with 1.2 ml of 0.9% saline with 0.1% Tween 20, and then incubated overnight at room temperature with 100 μl of ^{125}I-labelled rabbit anti-human IgE (Pharmacia UpJohn, Upsala, Sweden). The discs were washed four times and the bound ^{125}I was measured on a gamma counter (Packard, Berkshire, UK). All the assays were performed in duplicate. The results were expressed as a RAST% binding that was defined as the RAST% binding for the colophony–cell protein conjugate disc, minus the cell protein disc.

Results

Clinical data from the exposed symptomatic population are shown in Table 1. Subjects were engaged in a range of jobs involving work with colophony. All reported work-related respiratory symptoms, and six out of seven were clinically characterized in more detail. While these represent a mixed group, all were clearly exposed and reported work-related respiratory symptoms. As such, the likelihood of occupational asthma is high in this group. They were not all clinically characterized to the same degree.

IgE binding to the four colophony conjugates is shown in Table 2. Due to small stocks of patient serum from some subjects, it was not possible to test some samples to all four colophony–cell conjugates. Taking results from individuals who had increased binding of >0% to one or more of the colophony–cell conjugates, it can be seen that 86% (6/7) of samples tested in the symptomatic group were positive compared with both the exposed non-symptomatic [20% (2/10) of samples tested] and the non-exposed control populations [27% (3/11) of samples tested].

If a more conservative cut-off (>0.1%) is used to discriminate between a positive and a negative result, individuals who had increased binding to one or more of the colophony–cell conjugates represent 29% (2/7) of samples tested in the symptomatic group compared with both the exposed non-symptomatic and the non-exposed control populations (both 0% of samples tested).

Table 2. RAST% binding for individuals in each group (after correction for binding to cells alone) to a variety of colophony–cell conjugates

	RAST% binding			
	Portuguese	Fisons PA	BDH resin	American
Exposed non-symptomatic				
1	0	0	0	0
2	0.01	0	0	0
3	0	0	0	0
4	0	0	–	0
5	0	0	0	0
6	0	0	0	0
7	0	0	0	0
8	0	0	0	0
9	0	0.03	0	0
10	0	0	0	0
Non-exposed control				
1	0	0	0	0.01
2	0	–	0	0.03
3	–	0	0	0
4	0	0	0	0.03
5	0	0	0	0
6	–	0	0	0
7	–	0	–	0
8	–	0	0	0
9	–	0	0	0
10	0	0	0	0
11	0	0	–	0
Exposed symptomatic				
1	0.02	0.1	0.18	0.18
2	0	–	0	0.06
3	0.12	0.16	0	0
4	0	0	0	0
5	0.05	0	0.06	0
6	0.05	–	0.01	0.02
7	0.03	0.01	0	0

Dashed line indicates insufficient serum to carry out test.

Discussion

Occupational asthma induced by the inhalation of fumes from heated colophony as part of the soldering flux has been well documented in the electronics industry. Abietic acid is one of the main constituents of colophony, and this has been implicated as the likely cause of occupational asthma [14] and contact allergy.

It has been demonstrated that abietic acid can become oxidized in air to form resin acid epoxides and hydroperoxides that have now been suggested to be the sensitizer in contact allergy [15,16]. During the soldering process, colophony is heated to temperatures which we have shown would result in the degradation of any existing epoxides or hydroperoxides. It is unlikely that the pyrolysis fumes inhaled by the exposed workers would contain the oxidation products of abietic acid which would reasonably suggest that these oxidative products are not the causative agents of the colophony-induced asthma. We have previously demonstrated increased intracellular peroxide within immune cells challenged with colophony [11]. The stimulation of an oxidative burst by colophony led us to hypothesize that the oxidation of abietic acid may occur *in vivo* due to the oxidative products produced by the stimulation of monocytes and macrophages in the airways.

Furthermore, these oxidation products, which may be epoxides or hydroperoxides, could interact with the body protein to further initiate immune responses. The data presented in this report substantiate this hypothesis, since we developed a novel *in vitro* approach for the production of reagents for use in RAST analysis utilizing the ability of colophony to induce the production of reactive oxygen species in immune cells.

Cell colophony conjugates produced in this way were able to detect increased binding of specific IgE antibody in serum from individuals who were exposed, symptomatic and have a high likelihood of occupational asthma (supported by clinical evidence). Although the number of serum samples was small, conjugates produced from a range of colophony sources were able to bind specific IgE antibodies from this symptomatic group.

Results were used from individuals from either of the three groups who showed increased binding to one or more of the colophony–cell conjugates, as the similar protein structures of the different colophony species may cross-react with one another, resulting in 'false-positive' outcomes. For the purposes of this study, two different cut-offs have been used to report binding of colophony to specific IgE. In the first instance, a RAST% binding of $>0\%$ was used. In this case, assuming that all seven of the exposed symptomatic subjects tested have occupational asthma, the diagnostic sensitivity and specificity of the assay is calculated as 86 and 76%, respectively. A more conservative cut-off of $>0.1\%$ was also reported, resulting in a sensitivity and specificity of 29 and 100%, respectively.

Clearly, additional work is required to confirm that the increased binding observed in this study is due to an immunological process. Characterizing the allergen–antibody complex (for example, by immunoblot) and conducting dose–response studies on sera from sensitive individuals would provide valuable additional information to support the findings reported here. The possibility of 'false-negative' results should also be considered. Levels of allergen-specific IgE may fluctuate to undetectable levels in an individual with time and exposure yet the individual may still remain clinically allergic. In the exposed symptomatic group, subject number 4 had a negative RAST result in the presence of a strong clinical history, prompting the need for further investigation.

In conclusion, we suggest that the approach that we have used to produce conjugates for use in RAST using colophony as a model substance (i.e. utilizing the induction of oxidative burst in immune cells following *in vitro* challenge) offers an alternative approach for the investigation of sensitization to potential low molecular weight sensitizers.

References

1. Ross DJ, Sallie BA, McDonald JC. SWORD'94: surveillance of work-related and occupational respiratory disease in the UK. *Occup Med (Lond)* 1995;**45:**175–178.
2. Courtney D, Merret JD. Respiratory symptoms and lung function in a group of solderers. *Br J Ind Med* 1984;**41:** 346–351.
3. Lee HS, Koh D, Chia HP, Phoon WH. Symptoms, lung function, and diurnal variation in peak expiratory flow rate among female solderers in the electronics industry. *Am J Ind Med* 1994;**26:**613–619.
4. Palmer K, Crane G. Respiratory disease in workers exposed to colophony solder flux fumes: continuing health concerns. *Occup Med (Lond)* 1997;**47:**491–496.
5. Burge PS, Edge G, Hawkins R, White V, Newman Taylor AJ. Occupational asthma in a factory making flux-cored solder. *Thorax* 1981;**36:**828–834.
6. Burge PS, Perks WH, O'Brien IM, Burge A, Hawkins R, Brown D, Green M. Occupational asthma in an electronics factory; a case control study to evaluate aetiological factors. *Thorax* 1979;**34:**300–307.
7. Burge P. Occupational asthma in electronics workers caused by colophony fumes: follow-up of affected workers. *Thorax* 1982;**37:**348–353.
8. Fawcett I, Newman Taylor AJ, Pepys J. Asthma due to inhaled chemical agents fumes from 'Multicore' soldering flux and colophony resin. *Clin Allergy* 1976;**6:** 577–585.
9. Burge PS, Harries MG, O'Brien IM, Pepys J. Bronchial provocation studies in workers exposed to the fumes of electronic soldering fluxes. *Clin Allergy* 1980;**10:**137–149.
10. Burge PS, Wieland A, Robertson AS, Weir D. Occupational asthma due to unheated colophony. *Br J Ind Dis* 1986;**43:** 559–560.
11. Elms J, Allan LJ, Pengelly I, Fishwick D, Beckett PN, Curran AD. Colophony: an *in vitro* model for the induction of sensitization. *Clin Exp Allergy* 2000;**30:**209–213.
12. Elms J, Beckett PN, Griffin P, Curran AD. Mechanisms of isocyanate sensitisation. An *in vitro* approach. *Toxicol In Vitro* 2001;**15:**631–634.
13. Gannon PF, Newton DT, Belcher J, Pantin CF, Burge PS. Development of OASYS-2: a system for the analysis of serial measurement of peak expiratory flow in workers with suspected occupational asthma. *Thorax* 1996;**51:**484–489.
14. Cullen RT, Cherie B, Soutar C. Immune responses to colophony, an agent causing occupational asthma. *Thorax* 1992;**47:**1050–1055.
15. Gäfvert E, Shao LP, Karlberg AT, Nilsson U, Nilsson JLG. Contact allergy to resin acid hydroperoxides. Hapten binding via free radicals and epoxides. *Chem Res Toxicol* 1994;**7:**260–266.
16. Karlberg A-T, Bohlinder K, Boman A, Hacksell U, Hermansson J, Jacobsson S, Nilsson JLG. Identification of 15-hydroperoxyabietic acid as a contact allergen in Portuguese colophony. *J Pharm Pharmacol* 1988;**40:**42–47.