

Comparison of cytokine secretion profiles provoked in mice by glutaraldehyde and formaldehyde

R. J. DEARMAN*, D. A. BASKETTER†, P. EVANS‡ and I. KIMBER*

*Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, †Unilever Safety and Environmental Assurance Centre, Colworth House, Sharnbrook and ‡Health and Safety Executive, Magdalen House, Bootle, UK

Summary

Background Glutaraldehyde and formaldehyde are commonly used sterilizing agents that are known skin sensitizers. There is some controversy, however, regarding their capacity to cause respiratory allergy. The authors have demonstrated previously that topical exposure of mice to chemical contact allergens such as 2,4-dinitrochlorobenzene (DNCB) or respiratory allergens such as trimellitic anhydride (TMA) induces characteristic cytokine secretion profiles consistent with the selective activation of T helper 1 (TH1)- and TH2-type cells, respectively.

Objective To investigate the quality of immune response provoked following topical exposure of mice to these materials.

Methods BALB/c strain mice were exposed topically to 50% formaldehyde or to various concentrations of glutaraldehyde in acetone. Control animals were treated concurrently with the reference contact allergen DNCB (1% in acetone:olive oil [AOO]) or with the reference respiratory sensitizer TMA (10% in AOO). Thirteen days after the initiation of exposure, draining lymph node cells (LNCs) were cultured for 12–120 h and cytokine content of supernatants analysed by cytokine-specific enzyme-linked immunosorbent assay.

Results DNCB- α and formaldehyde-activated LNCs produced high levels of the TH1-type cytokine interferon γ , but little of the TH2-type products interleukins 4 and 10. TMA- and glutaraldehyde-stimulated LNCs displayed the converse TH2-type pattern of cytokine expression.

Conclusions These data are consistent with glutaraldehyde, but not formaldehyde, having significant potential to cause allergic sensitization of the respiratory tract.

Keywords: glutaraldehyde, formaldehyde, contact sensitization, respiratory sensitization, T lymphocytes, interleukin 4, interleukin 10, interferon- γ

Clinical and Experimental Allergy, Vol. 29, pp. 124–132. Submitted 4 March 1998; revised 29 April 1998; accepted 10 June 1998.

Introduction

Glutaraldehyde and formaldehyde have widespread applications in industry and the health sector; in the former as biocides and in the latter as biocides and preservatives. While both chemicals are recognized as causes of allergic contact dermatitis [1,2], their potential to induce occupational asthma is more controversial. One confounding factor is that both materials are highly irritating to the eyes, skin and respiratory system, making difficult differentiation

between irritant and allergic reactions [3,4]. A body of evidence has accumulated to suggest that glutaraldehyde is a significant cause of occupational asthma. Thus, in seven out of eight workers with suspected glutaraldehyde-related respiratory symptoms, specific bronchial responses could be provoked [5], with positive workplace reactions observed also in individual case studies of symptomatic patients [6,7]. There is some limited evidence that such respiratory responses to glutaraldehyde may be mediated via IgE antibody-dependent mechanisms, with specific IgE antibody detected in four out of 20 cases with respiratory symptoms attributed to this chemical [8]. Despite extensive exposure to formaldehyde, with estimates of up to 1.3 million exposed

Correspondence: R. J. Dearman, Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield SK10 4TJ, UK.

workers in the United States per annum [9], there are relatively few reported cases of occupational asthma to this chemical. Although positive specific bronchial provocation tests have been recorded for individuals occupationally exposed to formaldehyde [10–12], in the majority of cases negative challenge tests have been observed [11,13]. Moreover, several double-blind studies which incorporated intermittent exercise during challenge with formaldehyde were found not to affect parameters such as airways resistance [14,15]. Analyses of the serum of affected individuals for the presence of formaldehyde-specific IgE antibody have also resulted in equivocal findings, with occasional positive results which do not usually correlate with respiratory symptoms [16–18]. Indeed, it has been concluded previously that, taking into account the available evidence, even at relatively high exposure levels, inhaled formaldehyde rarely induces a specific IgE response and the relationship between this response and respiratory symptoms is weak [19]. Furthermore, it has been concluded recently that there is currently insufficient evidence to classify formaldehyde as a cause of asthma, as judged by the official European Union classification criteria [20].

At present there exist no widely accepted or validated experimental methods for the identification of chemicals that have the potential to cause sensitization of the respiratory tract [21]. One approach has been to characterize respiratory allergic activity as a function of inhalation-induced pulmonary reactions in previously sensitized animals, primarily using guinea-pigs [22]. This study has focused instead upon events which occur during the induction phase of respiratory sensitization. The production of IgE antibodies and the development of respiratory allergic reactions are associated with the activation of T helper (TH) 2 type cells and elevated expression of their cytokine products, including interleukins (IL) 4, 5 and 10. Indeed IL-4 is essential for the induction and maintenance of IgE antibody responses [23,24]. IL-4 gene-deleted mice fail to produce IgE antibodies under conditions which normally result in strong IgE responses [25]. Furthermore, the production of IL-5, acting possibly in concert with IL-4 [26], promotes the accumulation and activation of eosinophils in the airways [26,27], this being a characteristic feature of allergic hypersensitivity reactions of the respiratory tract [28]. While there is some controversy regarding a universal obligatory role for IgE in chemically induced respiratory allergy, particularly for isocyanate-induced asthma [29], responses of this type are associated usually with TH2-type cells, and the increased expression of the type 2 cytokines IL-4 and IL-5 [30,31]. Type 2 responses, such as IgE production and eosinophil accumulation, are antagonized by interferon- γ (IFN- γ) [32,33], a cytokine product of type 1 cells [34]. Type 1 cells and their soluble products play important roles in delayed type hypersensitivity responses such as contact

allergy [35,36]. The authors have shown previously that topical exposure of mice to chemical contact allergens such as dinitrochlorobenzene (DNCB) or to respiratory allergens such as trimellitic anhydride (TMA) induces divergent immune responses consistent with differential stimulation of discrete T-lymphocyte subpopulations. Lymph node cells (LNCs) isolated from BALB/c strain mice following repeated topical exposure to TMA express substantial amounts of the type 2 cytokines IL-4 and IL-10, but little of the type 1 product IFN- γ . In contrast, the converse cytokine secretion profile was provoked by exposure to the contact allergen DNCB [37,38]. These results suggest that it may be possible to characterize the nature of allergic disease a chemical will elicit as a function of the selectivity of induced cytokine production patterns stimulated in mice.

This approach was employed to compare the ability of formaldehyde and glutaraldehyde to stimulate cytokine production by draining LNCs following topical exposure of mice. Cytokine secretion profiles have been compared with those provoked in animals treated concurrently with the reference respiratory allergen TMA, or with the contact allergen DNCB.

Materials and methods

Animals

Young adult (6–12-week-old) female BALB/c strain mice (Harlan Seralab, Oxon, UK) were used throughout these studies.

Chemicals

Formalin (37% formaldehyde; this solution is referred to hereafter as formaldehyde) and glutaraldehyde (50% in aqueous solution) were from Sigma Chemical Co. (St Louis, MO, USA). All concentrations of these chemicals referred to subsequently in the text relate to these starting materials. 2,4-Dinitrochlorobenzene (DNCB; 98% pure) was obtained from Sigma and trimellitic anhydride (TMA; 97% pure) from Aldrich Chemical Co. (Gillingham, Dorset, UK). TMA and DNCB were dissolved in 4:1 acetone:olive oil (AOO), while glutaraldehyde and formaldehyde solutions were prepared with acetone.

Local lymph node assay

The local lymph node assay was conducted as described previously [39] with the exception that BALB/c strain mice were used. Groups of mice ($n = 4$) were exposed topically on the dorsum of both ears to 25 μ L of various concentrations of formaldehyde or glutaraldehyde, dissolved in acetone, or to an equal volume of vehicle alone, daily for 3 consecutive

days. Five days after the initiation of exposure, all mice were injected intravenously via the tail vein with 20 μ Ci of [^{3} H]-methyl thymidine (^{3} HTdR; specific activity 2 Ci/mmol; Amersham International, Amersham, UK) in 250 μ L of phosphate buffered saline (PBS). Five hours later, mice were killed and the draining auricular lymph nodes excised and pooled for each experimental group. A single cell suspension of lymph node cells (LNCs) was prepared by gentle mechanical disaggregation through 200-mesh stainless steel gauze. LNCs were washed twice with an excess of PBS and precipitated in 5% trichloroacetic acid (TCA). Twelve hours later, pellets were resuspended with 1 mL of TCA and transferred to 10 mL of scintillation fluid (Optiphase MP, LKB, UK). Incorporation of ^{3} HTdR was measured by β -scintillation counting and expressed as mean disintegrations per minute (d.p.m.) per node for each experimental group. Increases in ^{3} HTdR incorporation relative to vehicle-treated controls were derived for each experimental group and recorded as stimulation indices.

Sensitization of mice for cytokine production

Groups of mice ($n = 5$ for test chemicals, $n = 10$ for vehicles) received 50 μ L of 50% formaldehyde or various concentrations of glutaraldehyde, or vehicle (acetone) alone, bilaterally on each shaved flank. Groups of control animals were treated concurrently with 10% TMA or 1% DNCB dissolved in AOO, or with AOO vehicle alone. Five days later treatment was repeated. After a further 5 days, 25 μ L of chemical was applied to the dorsum of both ears daily, for 3 consecutive days.

Preparation of cells from draining lymph nodes

Thirteen days after the initial exposure, draining auricular lymph nodes were excised and pooled for each experimental group. A single cell suspension of LNCs was prepared under aseptic conditions by mechanical disaggregation through sterile 200-mesh stainless steel gauze. Viable cell counts were performed by exclusion of 0.5% trypan blue and LNCs were cultured in RPMI-1640 growth medium (Gibco, Renfrewshire, UK) supplemented with 25 mmol/L HEPES, 400 μ g/mL streptomycin, 400 μ g/mL ampicillin and 10% heat-inactivated fetal calf serum (RPMI-FCS). Cells (1 mL) at 10^7 cells/mL were seeded into 24-well tissue culture plates and maintained at 37°C in a humidified atmosphere of 5% CO₂ in air in the presence or absence of 2 μ g/mL concanavalin A (con A; Sigma). Culture was terminated after 12–120 h, supernatants collected, centrifuged at 100 *g* for 5 min and stored at –70°C prior to analysis.

Cytokine determinations

IL-4 The IL-4 content of culture supernatants derived

from con A-stimulated LNCs was measured by sandwich enzyme-linked immunosorbent assay (ELISA) as described previously [37]. Plastic microtitre plates (Nunc, Copenhagen, Denmark) were coated by overnight incubation at 4°C with 2.5 μ g/mL rat monoclonal anti-mouse IL-4 antibody (Genzyme, Cambridge, MA, USA) in 0.1 mol/L carbonate buffer (pH 9.6). The plates were blocked for 30 min at 37°C with 5% FCS in PBS. Recombinant murine IL-4 ($1\text{--}2 \times 10^7$ U/mg; Genzyme) diluted in RPMI-FCS and samples of conditioned medium diluted to various extents in RPMI-FCS were added to triplicate wells and the plates incubated for 2 h at room temperature. Plates were then incubated for 2 h at room temperature with 8 μ g/mL of goat anti-mouse IL-4 (R & D Systems Europe, Abingdon, Oxon, UK) and for a further 2 h at room temperature with a 1:500 dilution in RPMI-FCS of peroxidase-conjugated donkey anti-goat IgG (Serotec, Kidlington, UK). Enzyme substrate (o-phenylenediamine and urea hydrogen peroxide) was added and the reaction terminated after 15 min by addition of 0.5 mol/L citric acid. Between each incubation stage the plates were washed with PBS containing 0.05% Tween 20. Optical density at 450 nm was measured using an automated reader (Multiskan, Flow Laboratories, Irvine, Ayrshire, UK). A standard curve derived with recombinant murine IL-4 was used to calculate the IL-4 concentration in supernatants. The limit of detection was 600 pg/mL. Samples were analysed in triplicate, SD were <10% in most experiments.

IFN- γ

A sandwich ELISA was used to measure IFN- γ content of culture supernatants. Plastic microtitre plates (Nunc) were coated by overnight incubation at 4°C with 1 μ g/mL hamster monoclonal anti-mouse IFN- γ (Genzyme) in 0.1 mol/L carbonate buffer (pH 9.6). The plates were blocked for 30 min at 37°C with 5% FCS in PBS. Recombinant murine IFN- γ (Genzyme; 10^7 U/mg) diluted in RPMI-FCS and samples of conditioned medium diluted to various extents in RPMI-FCS were added to triplicate wells and the plates incubated for 2 h at room temperature. The plates were then incubated for 2 h at room temperature with 4 μ g/mL goat anti-mouse IFN- γ (Genzyme) diluted in RPMI-FCS and a further 2 h at room temperature with a 1:500 dilution of peroxidase-conjugated donkey anti-goat IgG (Serotec). Enzyme substrate (o-phenylenediamine and urea hydrogen peroxide) was added and the reaction terminated after 15 min by the addition of 0.5 mol/L citric acid. Between each incubation stage the plates were washed as described above. Optical density at 450 nm was measured using an automated reader (Multiskan). The concentration of IFN- γ in culture supernatants was calculated from a standard curve derived with recombinant murine IFN- γ .

The limit of detection of IFN- γ was 150 pg/mL. Standard deviations were <10% in most experiments.

IL-10 The IL-10 content supernatants was analysed using dual-monoclonal sandwich ELISA. Plastic microtitre plates were coated with 2 μ g/mL rat monoclonal anti-mouse IL-10 antibody (Pharmingen, San Diego, CA, USA) in 0.1 mol/L carbonate buffer (pH 9.6) by overnight incubation at 4°C. The plates were then blocked by treatment for 2 h at room temperature with 10% FCS in PBS. Recombinant mouse IL-10 (Genzyme; 5×10^5 U/mg) diluted in RPMI-FCS and samples of LNC-conditioned culture medium diluted to various extents in RPMI-FCS were added to triplicate wells and the plates incubated for 4 h at room temperature. Plates were then incubated for 1 h 30 min at room temperature with 2 μ g/mL biotinylated rat anti-mouse IL-10 (Pharmingen) and for a further 1 h at room temperature with 2.5 μ g/mL Extr-Avidin peroxidase (Sigma) diluted in 10% FCS in PBS. Enzyme substrate (tetramethyl benzidine and hydrogen peroxide) was added and the reaction terminated after 10 min by the addition of 2 mol/L sulphuric acid. Between each incubation, the plates were washed as described above. Optical density at 450 nm was measured using an automated reader (Multiskan). The concentration of IL-10 in culture supernatants was calculated from a standard curve derived with recombinant murine IL-10. The limit of detection was 300 pg/mL. Standard deviations were <10% in most experiments.

Results

In preliminary experiments it was confirmed that both formaldehyde and glutaraldehyde possess contact-sensitizing activity. The murine local lymph node assay, a test which identifies contact allergens as a function of lymphocyte proliferative responses induced in the lymph node draining the site of topical exposure, was performed as described

Fig. 1. Groups of mice ($n = 4$) received 25 μ L of various concentrations of formaldehyde (HCHO) (a) or glutaraldehyde (glu.) (b) in acetone vehicle, or an equal volume of vehicle alone, on the dorsum of both ears, daily for 3 consecutive days. Five days following the initiation of treatment all mice were injected intravenously with 250 μ L PBS containing 20 μ Ci ^{3} HTdR. Draining auricular lymph nodes were excised 5 h later, pooled for each experimental group and a single cell suspension of LNCs prepared. Increases in incorporation of ^{3} HTdR relative to vehicle-treated controls were derived for each experimental group and displayed graphically as stimulation indices. The dashed line represents a stimulation index of 3. d.p.m./node values are as follows: acetone 165 d.p.m./node; 1% HCHO 132 d.p.m./node; 5% HCHO 294 d.p.m./node; 10% HCHO 576 d.p.m./node; 25% HCHO 866 d.p.m./node; 50% HCHO 1243 d.p.m./node; 0.5% glu. 300 d.p.m./node; 1% glu. 489 d.p.m./node; 5% glu. 2431 d.p.m./node; 10% glu. 2724 d.p.m./node; 15% glu. 4568 d.p.m./node.

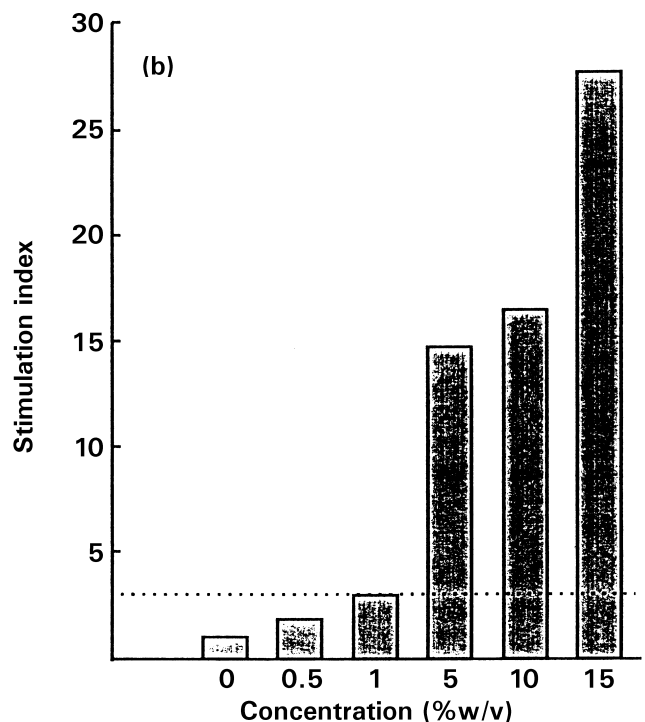
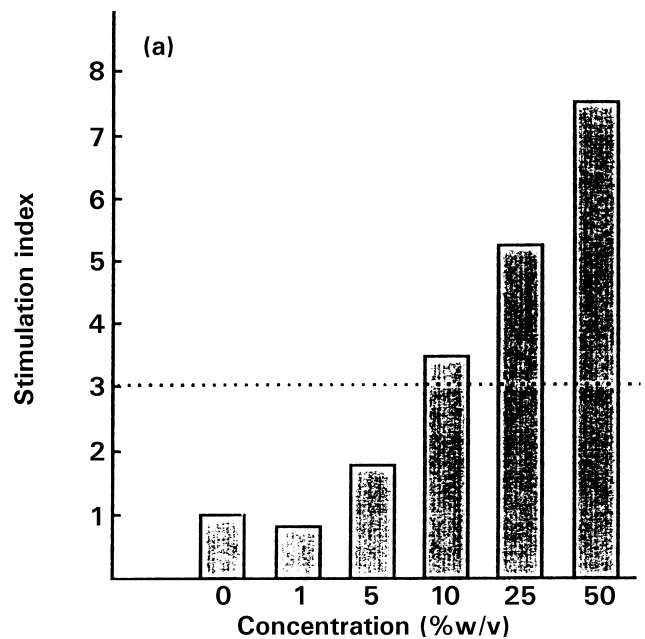


Table 1. Peak cytokine production following topical exposure to formaldehyde and glutaraldehyde

Treatment	IL-4 concentration (ng/mL)						IL-10 concentration (ng/mL)						IFN- γ concentration (ng/mL)					
	Expt 1	Expt 2	Expt 3	Expt 4	Expt 5	Mean $\pm$ SE	Expt 1	Expt 2	Expt 3	Expt 4	Expt 5	Mean $\pm$ SE	Expt 1	Expt 2	Expt 3	Expt 4	Expt 5	Mean $\pm$ SE
TMA	2.8	25.0	2.0	4.8	4.8	7.88 $\pm$ 4.33	1.7	1.35	1.45	0.95	1.15	1.28 $\pm$ 0.12	0.32	0.33	0.15	<0.15	0.96	0.38 $\pm$ 0.15
DNCB	<0.6	0.8	<0.6	<0.6	<0.6	0.64 $\pm$ 0.04	<0.3	<0.3	<0.3	<0.3	<0.3	–	2.85	8.0	3.9	5.15	6.0	5.18 $\pm$ 0.88
50% HCHO	<0.6	4.0	<0.6	<0.6	<0.6	1.28 $\pm$ 0.68	0.9	<0.3	<0.3	0.58	<0.3	0.66 $\pm$ 0.18	5.1	8.0	10.0	8.8	28.0	11.98 $\pm$ 5.35
15% glu.	0.8	15.5	1.25	<0.6	1.15	3.86 $\pm$ 2.91	0.92	1.55	1.7	1.05	1.1	1.17 $\pm$ 0.15	0.88	1.4	1.9	2.2	6.6	2.60 $\pm$ 1.02
AOO	ND	ND	<0.6	<0.6	<0.6	–	ND	ND	<0.3	<0.3	<0.3	–	ND	ND	0.5	<0.15	2.1	1.25 $\pm$ 0.52
Acetone	ND	<0.6	<0.6	<0.6	<0.6	–	ND	<0.3	<0.3	<0.3	<0.3	–	ND	<0.15	2.7	<0.15	0.72	0.93 $\pm$ 0.70
Naives	ND	ND	ND	<0.6	<0.6	–	ND	ND	<0.3	<0.3	<0.3	–	ND	ND	<0.15	<0.15	2.15	0.82 $\pm$ 0.67

Groups of mice ($n = 5$ for chemical; $n = 10$ for vehicle) received 50 μ L of 10% TMA in AOO, 1% DNCB in AOO, 50% formaldehyde (HCHO) in acetone, 15% glutaraldehyde (glu.) in acetone, or vehicle alone, bilaterally on the shaved flanks. This treatment was repeated 5 days later and after a further 5 days, 25 μ L of chemical or vehicle alone was applied to the dorsum of both ears daily for 3 consecutive days. Further groups of mice ($n = 10$) were untreated (naive). Thirteen days following the initiation of exposure, draining auricular lymph nodes were excised, pooled for each experimental group and a single cell suspension of LNCs prepared under aseptic conditions. Peak cytokine concentrations (IL-4, 24 h; IL-10 and IFN- γ , 120 h) were measured by ELISA in supernatants prepared following culture of LNCs in 5 independent experiments. ND, not determined.

previously [39], with the exception that BALB/c, rather than CBACa, strain mice were used (Fig. 1). At the maximum (non-irritant) concentration tested, both formaldehyde (50%) and glutaraldehyde (15%) elicited positive responses in the local lymph node assay, according to the criterion of the induction of a threefold or greater increase in proliferative activity compared with concurrent vehicle-treated controls [39]. As reported previously [40], glutaraldehyde provoked a more vigorous response than did formaldehyde, with respect to both the maximal proliferative response elicited (a stimulation index of 27.7 compared with 7.5), and with respect to the lowest concentration of material found to induce a positive (threefold or greater stimulation index) response (1% glutaraldehyde compared with 10% formaldehyde).

In subsequent experiments, the patterns of cytokine production induced in draining LNCs were measured following repeated topical exposure of mice to 50% formaldehyde or to 15% glutaraldehyde in acetone vehicle or to vehicle alone. For comparative purposes, additional groups of mice were treated concurrently with 1% DNCB or 10% TMA in AOO vehicle to elicit, respectively, TH1- and TH2-type cytokine secretion profiles as described previously [37]. Further controls received AOO alone or were untreated (naive).

Peak mitogen-induced IL-4 production (24 h) and spontaneous IL-10 and IFN- γ secretion (120 h) for five independent experiments are displayed in Table 1. While there was some inter-experimental variation in the absolute amounts of cytokine produced, particularly for con A-stimulated IL-4 secretion, in each of the five independent experiments reported, and consistent with previous investigations [37], TMA-activated LNCs elaborated high levels of IL-10 (0.95–1.7 ng/mL) and mitogen-inducible IL-4 (2–25 ng/mL). Expression of the type 1 cytokine IFN- γ was low (0.15–0.96 ng/mL) or below the limit of detection (< 0.15 ng/mL). In contrast, in the majority of experiments, DNCB-stimulated LNCs failed to produce detectable levels of IL-4 or IL-10 (< 0.6 and 0.3 ng/mL, respectively), whereas a significant potential to secrete IFN- γ was observed (between 2.85 and 8.0 ng/mL). Treatment of mice with 50% formaldehyde resulted in a selective type 1 cytokine phenotype. Secretion of the type 2 cytokines IL-4 and IL-10 was generally below limits of detection, while IFN- γ production was vigorous (5.1–28 ng/mL), equalling or exceeding the levels of this cytokine produced by DNCB-stimulated LNCs. Exposure to 15% glutaraldehyde, however, stimulated a type 2 cytokine secretion profile. Similar levels of IL-10 (0.92–1.7 ng/mL) to those provoked by treatment with TMA were observed,

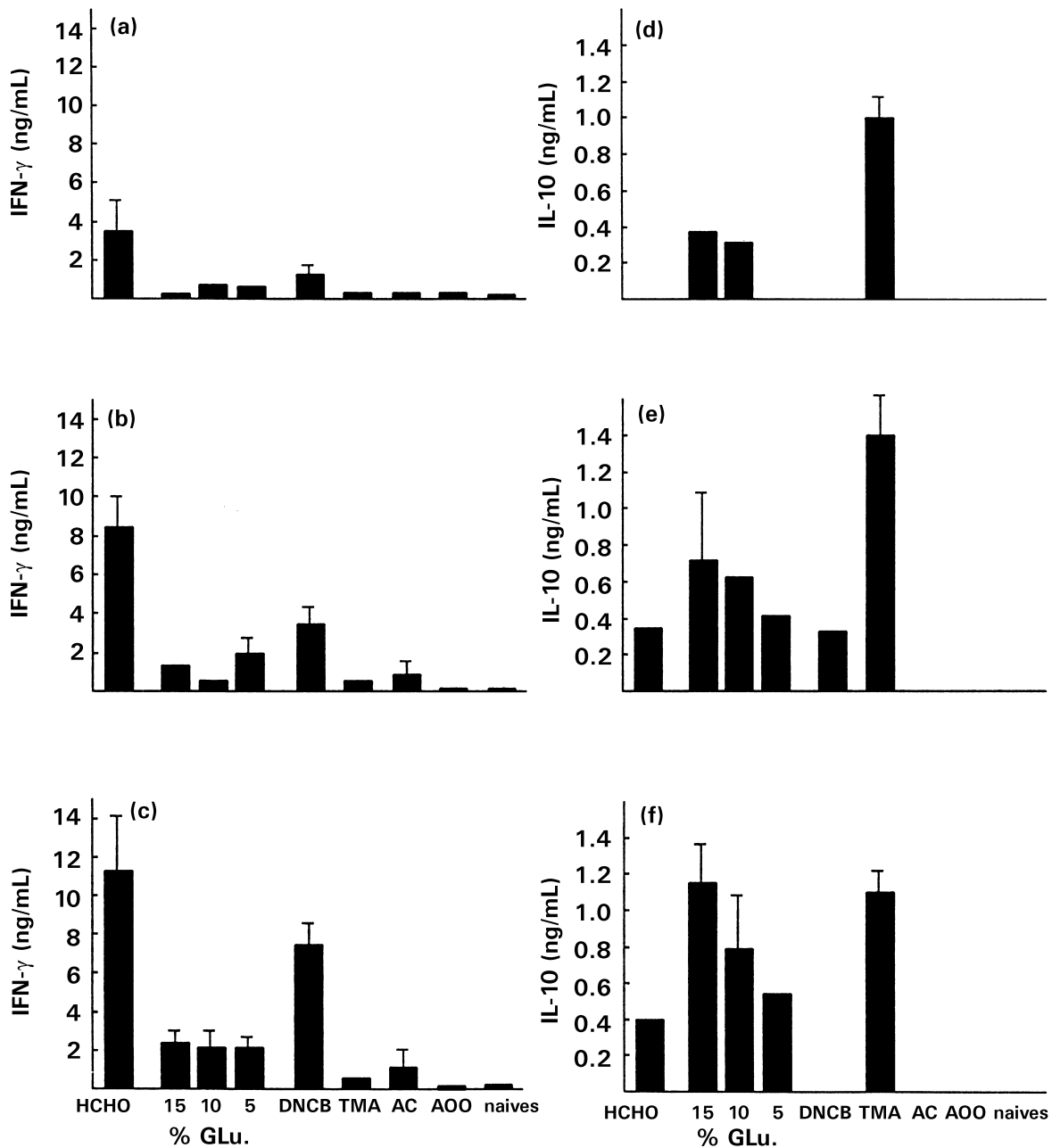


Fig. 2. Groups of mice ($n = 5$ for chemical; $n = 10$ for vehicle) received 50 μ L of 50% formaldehyde (HCHO) or various concentrations of glutaraldehyde (glu.) in acetone, 1% DNFB or 10% TMA in AOO, or vehicle alone, bilaterally on the shaved flanks. This treatment was repeated 5 days later and after a further 5 days, 25 μ L of chemical or vehicle alone was applied to the dorsum of both ears daily for 3 consecutive days. Further groups of mice ($n = 10$) were untreated (naive). Thirteen days after the initiation of exposure, draining auricular lymph nodes were excised, pooled for each experimental group and a single cell suspension of LNCs prepared under aseptic conditions. LNCs were cultured for 72 (a, d), 96 (b, e) and 120 h (c, f) and IFN- γ (a, b, c) and IL-10 (d, e, f) content of supernatants determined by ELISA. Mean cytokine concentrations $\pm$ SE are shown for 3 independent experiments where SE > 0.5 or 0.02 ng/mL (IFN- γ and IL-10, respectively). IL-10 levels for AOO- and acetone-treated animals and naive animals were all below the limits of detection.

whereas mitogen-inducible IL-4 production was somewhat less vigorous than seen with TMA (ranging from below the limit of detection to 15.5 ng/mL). In the majority of experiments, secretion of the type 1 product IFN- γ by

glutaraldehyde-activated LNCs was considerably lower than that expressed by formaldehyde- or DNFB-stimulated LNCs (0.88–6.6 ng/mL). LNCs prepared from naive or vehicle-treated animals failed to express measurable levels

of IL-10 or con A-inducible IL-4; with levels of IFN- γ being low or below the limit of detection (<0.15 ng/mL).

Finally, dose response analyses were performed with glutaraldehyde to determine whether changes in cytokine phenotype are stimulated at concentrations of chemical which result in similar levels of lymphocyte proliferation to those induced by 50% formaldehyde. Animals were treated with 15, 10 or 5% glutaraldehyde or with 50% formaldehyde in acetone vehicle or with vehicle alone. Concurrent control groups received 1% DNCB or 10% TMA in AOO vehicle, or AOO alone. Draining LNCs were cultured for 72, 96 and 120 h and supernatants analysed for IL-10 and IFN- γ content. Results (mean $\pm$ SE) of three independent experiments are shown in Fig. 2. Maximal production of both cytokines was induced after 120 h of culture. As observed in previous experiments (compare with Table 1), exposure to formaldehyde and DNCB resulted in little or no detectable IL-10 secretion, but vigorous IFN- γ production, particularly with the former chemical. The converse type 2 cytokine secretion pattern was displayed by TMA-activated LNCs. LNCs isolated from vehicle-treated or naive animals did not elaborate detectable levels of IL-10 and only low levels of IFN- γ were observed. Exposure to 15% glutaraldehyde stimulated comparable, maximal levels of IL-10 to those observed following treatment with TMA, while exposure to 10% and 5% glutaraldehyde provoked less vigorous IL-10 expression. However, at all concentrations of glutaraldehyde tested, levels of IFN- γ secretion were similar to those caused by exposure to acetone vehicle alone. IFN- γ concentrations measured in cultures of formaldehyde-stimulated LNCs were at least fivefold higher. Treatment with lower concentrations of glutaraldehyde (1%; one experiment) failed to stimulate measurable IL-10 production and levels of IFN- γ secretion were less than those observed following treatment with acetone vehicle alone (after 120 h culture, 1.85 ng/mL and 3.05 ng/mL, respectively; concurrent DNCB and formaldehyde controls were 7.0 ng/mL and 17.0 ng/mL, respectively).

Discussion

In agreement with previous results from experimental investigations in animals [40], the data from the present studies confirm that both formaldehyde and glutaraldehyde exhibit the capacity to induce contact sensitization. Consistent with occupational health experience, the latter material displayed a considerably greater potential to stimulate skin sensitization than did formaldehyde [1,2]. Analysis of the induced cytokine secretion profiles of allergen-activated LNCs revealed, however, that these materials provoke divergent immune responses suggestive of the selective activation of discrete functional subpopulations of T lymphocytes. As described previously [37,38], formaldehyde-stimulated LNCs expressed high levels of the TH1-type cytokine IFN- γ ,

but little of the TH2-type products IL-4 or IL-10; a pattern identical to that induced by the reference contact allergen DNCB. Exposure to glutaraldehyde resulted in the converse TH2-type profile, comparable with responses elicited by the reference respiratory sensitizer TMA. The discrete cytokine phenotypes provoked by formaldehyde and glutaraldehyde were not simply a function of the differential immunogenicity of these materials. Concentrations of glutaraldehyde that induced similar levels of immunogenicity to formaldehyde with respect to lymphocyte proliferative responses stimulated a TH2-type pattern of cytokine expression or provoked similar cytokine production profiles to those observed following exposure to vehicle (acetone) alone. Taken together, the experimental evidence is consistent with glutaraldehyde, but not formaldehyde, having the potential to induce the quality of immune activation necessary for the production of IgE antibody and the development of respiratory sensitization. As such, these data are in agreement with the results of a previous investigation by Potter and Wederbrand in which it was demonstrated that topical exposure of BALB/c mice to the former, but not to the latter, chemical resulted in a significant elevation in the concentration of total serum IgE [41]. It is important to note that even in the absence of the production of specific IgE antibody, TH2-type cells and their cytokine products may well directly promote the development of allergic hypersensitivity reactions of the respiratory tract. Airways inflammation and asthma may be induced by the direct action of IL-5 (possibly acting in concert with IL-4) on eosinophil accumulation and activation [26,27,40]. The induction of a preferential TH1-type response by formaldehyde is compatible with the possession by this chemical of skin-sensitizing activity. The experimental evidence is therefore supportive of the conclusions from epidemiological data that while glutaraldehyde exhibits a potential to cause allergic sensitization of the respiratory tract, formaldehyde, even at relatively high exposure levels, apparently lacks the ability to induce respiratory allergy [5,8,19,20].

While the selective cytokine secretion profiles induced following topical exposure of mice to different classes of chemical allergen are indicative of the development of discrete functional subpopulations of CD4⁺ TH cells, there is increasing evidence that other lymphocyte populations may also contribute to the induced cytokine phenotype. A similar functional heterogeneity has been found among CD8⁺ lymphocytes, with two main subsets, designated TC1 and TC2, exhibiting cytokine selectivity comparable with TH1 and TH2 cells, respectively [42,43]. There is evidence that both TC and TH cell subsets contribute to the cytokine secretion patterns displayed by murine allergen-activated LNCs. Thus, the high levels of IFN- γ associated with exposure to materials such as DNCB and formaldehyde derived from both CD4⁺ and CD8⁺ cells, while the low

levels of this cytokine provoked by respiratory allergens such as TMA were associated exclusively with CD8⁺ TC1 cells. The vigorous production of the type 2 cytokines induced by respiratory sensitizers, however, was found to derive solely from TH2 CD4⁺ cells [44]. Therefore, it would seem that not only does repeated topical exposure of mice to chemical respiratory and contact allergens result in divergent cytokine phenotypes but also that such patterns of cytokine secretion are a function of differential development of functional subpopulations of both TH and TC cells. Regardless of the cellular source of cytokines, however, it is clear that the type 1 pattern induced by formaldehyde is unlikely to be permissive for the production of IgE.

An important consideration is the fact that in the experiments described here, a differential quality of immune response is induced following cutaneous exposure to formaldehyde or glutaraldehyde. As such, the preferential activation of type 1, IFN- γ -secreting, LNCs and the failure to elicit increases in the total serum concentration of IgE following topical application of formaldehyde to mice [38,41], are consistent with the inability to detect specific IgE antibody in patients with formaldehyde-induced atopic dermatitis [45]. There is good reason to suppose that, due to the systemic nature of the immune system, topical exposure to chemical respiratory allergens can result in effective sensitization of the respiratory tract. There is some evidence, however, that the route of exposure may affect the balance between TH1- and TH2-type responses. Thus, it has been suggested that in lymph nodes draining mucosal tissues the development of TH2-type immune responses is favoured, while in nodes draining non-mucosal sites TH1-type responses are predominant [46]. It seems probable that the route of exposure does not influence markedly the nature of immune responses induced in chemical allergens in mice. Thus, the contact allergen DNCB fails to provoke detectable IgE antibody production following exposure via either topical or inhalation routes, under conditions where IgG antibody responses are stimulated [47].

In agreement with the general consensus from clinical investigations [4–8, 10–15, 19,20], these data are consistent with glutaraldehyde, but not formaldehyde, having the potential to cause allergic sensitization of the respiratory tract. These studies suggest that the analysis of induced cytokine secretion profiles by activated LNCs may provide a means of distinguishing between materials with a potential to cause respiratory allergy and those which are primarily respiratory irritants.

Acknowledgements

This work was supported in part by a grant from the UK Health and Safety Executive.

References

- 1 Marzulli FN, Maibach HI. The use of graded concentrations in studying skin sensitizers: experimental contact sensitization in man. *Fd Cosmet Toxicol* 1974; 12:219–27.
- 2 Nethercott JR, Holness DL. Contact dermatitis in funeral service workers. *Contact Derm* 1988; 18:263–7.
- 3 Bardana EJ, Montanaro A. Formaldehyde: an analysis of its respiratory, cutaneous and immunologic effects. *Ann Allergy* 1991; 66:441–52.
- 4 Norback D. Skin and respiratory symptoms from exposure to alkaline glutaraldehyde in medical services. *Scand J Work Environ Health* 1988; 14:366–71.
- 5 Gannon PFG, Bright P, Campbell M, O'Hickey SP, Sherwood Burge P. Occupational asthma due to glutaraldehyde and formaldehyde in endoscopy and X ray departments. *Thorax* 1995; 50:156–9.
- 6 Cullinan P, Hayes J, Cannon J, Madan I, Heap D, Newman Taylor A. Occupational asthma in radiographers. *Lancet* 1992; 340:1477.
- 7 Chan-Yeung M, McMurren T, Catonio-Begley F, Lam S. Occupational asthma in a technologist exposed to glutaraldehyde. *J Allergy Clin Immunol* 1993; 91:974–8.
- 8 Curran AD, Burge PS, Wiley K. Clinical and immunologic evaluation of workers exposed to glutaraldehyde. *Allergy* 1996; 51:826–32.
- 9 Gough M, Hart R, Karrh BW et al. Report on the consensus workshop on formaldehyde. *Environ Health Perspectives* 1984; 58:323–81.
- 10 Hendrick DJ, Lane DJ. Occupational formalin asthma. *Br J Ind Med* 1977; 34:11–8.
- 11 Nordman H, Keskinen H, Tuppurainen M. Formaldehyde asthma — rare or overlooked? *J Allergy Clin Immunol* 1985; 75:91–9.
- 12 Burge PS, Harries MG, Lam WK, O'Brien IM, Pachett PA. Occupational asthma due to formaldehyde. *Thorax* 1985; 40: 255–60.
- 13 Frigas E, Filley WV, Reed CE. Bronchial challenge with formaldehyde gas — lack of bronchoconstriction in 13 patients suspected of having formaldehyde asthma. *Mayo Clin Pro* 1984; 59:295–9.
- 14 Witek TJ, Schacter EN, Tosun T, Beck GJ, Leaderer BP. An evaluation of respiratory effects following exposure to 2 p.p.m. formaldehyde in asthmatics: lung function, symptoms and airway reactivity. *Arch Env Health* 1987; 42:230–7.
- 15 Schachter EN, Witek TJ, Brody DJ et al. A study of respiratory effects from exposure to 2 p.p.m. formaldehyde in occupationally exposed workers. *Environ Res* 1987; 44:188–205.
- 16 Patterson R, Pateras V, Grammer LC, Harris KE. Human antibodies against formaldehyde-human serum albumin conjugates or human serum albumin in individuals exposed to formaldehyde. *Int Arch Appl Immunol* 1986; 79:53–9.
- 17 Kramps JA, Peltenburg LTC, Kerklaan PRM et al. Measurement of specific antibodies in individuals exposed to formaldehyde. *Clin Exp Allergy* 1989; 19:509–14.
- 18 Grammer LC, Harris KE, Cugell DW, Patterson R. Evaluation of a worker with possible formaldehyde-induced asthma. *J Allergy Clin Immunol* 1993; 92:29–33.

- 19 Smedley J. Is formaldehyde an important cause of allergic respiratory disease? *Clin Exp Allergy* 1996; 26:247–9.
- 20 Health and Safety Executive. *Asthmagen? Critical Assessments of the Evidence for Agents Implicated in Occupational Asthma*. Sudbury: HSE Books, 1997.
- 21 Selgrade MK, Zeiss CR, Karol MH et al. Workshop on status of test methods for assessing potential of chemicals to induce respiratory allergic reactions. *Inhal Toxicol* 1994; 6:303–19.
- 22 Karol MH. Animal models of occupational asthma. *Eur Respir J* 1994; 7:555–68.
- 23 Finkelman FD, Katona IM, Urban JF Jr et al. Suppression of in vivo polyclonal IgE responses by monoclonal antibody to the lymphokine B cell stimulatory factor 1. *Proc Natl Acad Sci USA* 1986; 83:9675–8.
- 24 Finkelman FD, Katona IM, Urban JF Jr et al. IL-4 is required to generate and sustain in vivo IgE responses. *J Immunol* 1988; 141:2335–41.
- 25 Kuhn R, Rajewsky K, Muller W. Generation and analysis of interleukin 4 deficient mice. *Science* 1991; 254:707–10.
- 26 Lukacs NW, Streiter RM, Chensue SW, Kunkel SL. Interleukin-4-dependent pulmonary eosinophil infiltration in a murine model of asthma. *Am J Resp Cell Mol Biol* 1994; 10:526–32.
- 27 Foster PS, Hogan SP, Ramsay AJ, Matthaei KI, Young IG. Interleukin 5 deficiency abolishes eosinophilia, airways hyper-reactivity and lung damage in a mouse asthma model. *J Exp Med* 1996; 183:195–201.
- 28 Metzger WJ, Zavala D, Richerson HB et al. Local allergen challenge and bronchoalveolar lavage of allergic asthmatic lungs: description of the model and local airway inflammation. *Am Rev Resp Dis* 1987; 135:433–40.
- 29 Chan-Yeung M. Occupational asthma. *Environ Health Perspectives* 1995; 103:249–52.
- 30 Bentley AM, Maestrelli P, Saetta M et al. Activated T-lymphocytes and eosinophils in the bronchial mucosa in isocyanate-induced asthma. *J All Clin Immunol* 1992; 89:821–9.
- 31 Maestrelli P, Occari P, Turato G et al. Expression of interleukin (IL)-4 and IL-5 proteins in asthma induced by toluene diisocyanate (TDI). *Clin Exp All* 1997; 27:1292–8.
- 32 Finkelman FD, Katona IM, Mosmann TR, Coffman RL. IFN- γ regulates the isotypes of Ig secreted during in vivo humoral immune responses. *J Immunol* 1988; 140:1022–7.
- 33 Iwamoto I, Nakajima H, Endo H, Yoshido S. Interferon γ regulates antigen-induced eosinophil recruitment into the mouse airways by inhibiting the infiltration of CD4⁺ T cells. *J Exp Med* 1993; 177:573–6.
- 34 Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol* 1986; 136:2348–57.
- 35 Cher DJ, Mosmann TR. Two types of murine helper T cell clone. II. Delayed-type hypersensitivity is mediated by Th1 clones. *J Immunol* 1987; 138:3688–94.
- 36 Diamanstein T, Eckert R, Volk H-D, Kupier-Weglinski J-W. Reversal by interferon- γ of inhibition of delayed-type hypersensitivity induction by anti-CD4 or anti-interleukin 2 receptor (CD25) monoclonal antibodies: Evidence for the physiological role of the CD4⁺ Th1⁺ subset in mice. *Eur J Immunol* 1988; 18:2101–3.
- 37 Dearman RJ, Smith S, Basketter DA, Kimber I. Classification of chemical allergens according to cytokine secretion profiles of murine lymph node cells. *J Appl Toxicol* 1997; 17:53–62.
- 38 Hilton J, Dearman RJ, Basketter DA, Scholes EW, Kimber I. Experimental assessment of the sensitizing properties of formaldehyde. *Fd Chem Toxic* 1996; 34:571–8.
- 39 Kimber I, Basketter DA. The murine local lymph node assay: a commentary on collaborative studies and new directions. *Fd Chem Toxic* 1992; 30:165–9.
- 40 Hilton J, Dearman RJ, Harvey P et al. Estimation of relative skin sensitizing potency using the local lymph node assay: a comparison of formaldehyde with glutaraldehyde. *Am J Contact Derm* 1998; 9:29–33.
- 41 Potter DW, Wederbrand KS. Total IgE antibody production in BALB/c mice after dermal exposure to chemicals. *Fund Appl Toxicol* 1995; 26:127–35.
- 42 Croft M, Carter L, Swain SL, Dutton RW. Generation of polarized antigen-specific CD8 effector populations: reciprocal action of interleukin (IL)-4 and IL-12 in promoting type 2 versus type 1 cytokine profiles. *J Exp Med* 1994; 180:1715–28.
- 43 Sad S, Marcotte R, Mosmann TR. Cytokine-induced differentiation of precursor mouse CD8⁺ T cells into cytotoxic CD8⁺ T cells secreting Th1 or Th2 cytokines. *Immunity* 1995; 2:271–9.
- 44 Dearman RJ, Moussavi A, Kemeny DM, Kimber I. Contribution of CD4⁺ and CD8⁺ T lymphocytes to the cytokine secretion patterns induced in mice during sensitization to contact and respiratory allergens. *Immunology* 1996; 89: 502–10.
- 45 Liden S, Scheynius A, Fischer T, Johansson SGO, Ruhnnek-Forsbeck M, Stejskal V. Absence of specific IgE antibodies in allergic contact sensitivity to formaldehyde. *Allergy* 1993; 48:525–9.
- 46 Daynes RA, Araneo BA, Dowell TA, Huang K, Dudley D. Regulation of murine lymphokine production in vivo. III. The lymphoid tissue microenvironment exerts regulatory influences over T helper cell function. *J Exp Med* 1990; 171:979–96.
- 47 Dearman RJ, Hegarty JM, Kimber I. Inhalation exposure of mice to trimellitic anhydride induces both IgG and IgE anti-hapten antibody. *Int Arch Allergy Appl Immunol* 1991; 95:70–6.