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Divergent immunological responses following glutaraldehyde exposure

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Abstract

Although Glutaraldehyde (Glut) has been demonstrated to be a moderate contact sensitizer, numerous cases of occupational asthma related to Glut exposure have been reported. The purpose of these studies was to examine the dose–response relationship between Glut exposure and the development of T cell-mediated vs. IgE- mediated responses. Initial evaluation of the sensitization potential was conducted using the local lymph node assay (LLNA) at concentrations ranging from 0.75% to 2.5%. A concentration-dependent increase in lymphocyte proliferation was observed with EC3 values of 0.072% and 0.089% in CBA and BALB/c mice, respectively. The mouse ear swelling test (MEST) was used to evaluate the potential for Glut to elicit IgE (1/2 h post challenge) and contact hypersensitivity (24 and 48 h post challenge) responses. An immediate response was observed in animals induced and challenged with 2.5% Glut, whereas animals induced with 0.1% or 0.75% and challenged with 2.5% exhibited a delayed response 48 h post challenge. IgE-inducing potential was evaluated by phenotypic analysis of draining lymph node cells and measurement of total serum IgE levels. Only the 2.5% exposed group demonstrated a significant increase ($P < 0.01$) in the percentage of $IgE^{+}B220^{+}$ cells and serum IgE. Following 3 days of dermal exposure, a significant increase in IL-4 mRNA in the draining lymph nodes was observed only in the 2.5% exposed group. These results indicate that the development of an immediate vs. a delayed hypersensitivity response following dermal exposure to Glut is at least in part mediated by the exposure concentration.

Keywords: Glutaraldehyde; Dose response; Th1/Th2 response; LLNA

Introduction

Glutaraldehyde (Glut) is an aliphatic dialdehyde that has a wide spectrum of industrial, scientific, and biomedical applications. It is used in health care settings as a disinfectant and sterilizing agent, in electron microscopy as a tissue fixative, in manufacturing of adhesives and sealants, and in many other processes. Widespread use of this chemical creates the potential for dermal, mucosal, and respiratory exposure. Glutaraldehyde is available in aqueous solutions in concentrations up to 50% v/v; however, most sterilizing solutions contain approximately 2% glutaraldehyde and even lower concentrations exist in the ambient air of sterilization rooms.

Depending on the nature of exposure, glutaraldehyde has been shown to induce irritant and immunologic effects. Exposure to glutaraldehyde well below occupational exposure limits (OSHA PEL and Swedish limit of 0.2 ppm) has been shown to induce eye, skin, and upper respiratory tract irritation as well as headache, nausea, and fatigue (Burge, 1989; Norback, 1988). Glutaraldehyde is also a moderate contact sensitizer (Ballantyne and Jordan, 2001; Curran et al., 1996; Goncalo et al., 1984; Kiec-Swierczynska and Krecisz, 2001; Nethercott et al., 1988; Shaffer and Belsito, 2000; Vyas et al., 2000). Additionally, numerous cases of occupational asthma resulting from glutaraldehyde exposure have been reported in recent years (Ballantyne and Jordan, 2001; Quirce et al., 1999; Stenton et al., 1994; Vyas et al., 2000).

Although many chemicals, including glutaraldehyde, have the potential to induce diverse immunological responses, the underlying factors are not well understood. The purpose of these studies was to evaluate the role that concentration of exposure plays in the devel-

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opment of T cell-mediated vs. IgE-mediated responses following dermal exposure to glutaraldehyde using a murine model.

Materials and methods

Animals. Female BALB/c, CBA, and B6C3F1 mice were purchased from Taconic (Germantown, NY) and Jackson Laboratories (Bar Harbor, ME), respectively. Animals were 6–8 weeks old upon arrival and were quarantined for at least 1 week before use. Mice were maintained under conditions specified by NIH guidelines (NIH, 1996), and were provided tap water and Agway Prolab Animal Diet (5% fat) ad libitum. Animal facilities were maintained between 18 and 26 °C and 25–70% relative humidity with light–dark cycles at 12-h intervals. Mice were weighed, tail-marked for identification, and assigned to homogeneous weight groups (n = 5 or 8) before each experiment. Body weights were recorded at the termination of each study.

Chemicals. Glutaraldehyde (grade I, 25% aqueous solution), *N*,*N*-dimethylformamide (DMF; purity 99.9%), 2,4-dinitrofluorobenzene (DNFB; purity 99.4%), and acetone (purity 100%) were all purchased from Sigma (St. Louis, MO). Alpha hexylcinnamaldehyde (HCA; purity 85%) was purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI). All chemicals were prepared in either acetone or DMF at concentrations presented in Table 1.

Local lymph node assay. The local lymph node assay (LLNA) was performed following the method described in the ICCVAM Peer Review Panel report (NIEHS, 1999) with minor modifications. Briefly, mice were exposed topically with vehicle (DMF), increasing concentrations of glutaraldehyde, or positive control (30% HCA) on the dorsal surface of each ear (25 μl per ear) for three consecutive days (Table 1). Animals were allowed to rest for 2 days following the last exposure. On day 6, mice were injected intravenously via the lateral tail vein with 20 μCi ^{3}H-thymidine (Dupont NEN; specific activity 2 Ci/mmol). Five hours after ^{3}H-thymidine injection, animals were euthanized via CO_2 inhalation, and the left and right cervical draining lymph nodes (DLNs) located at the bifurcation of the jugular vein were excised and pooled for each animal. Single cell suspensions were made and following overnight incubation in trichloroacetic acid (TCA), samples were counted using a Packard Tri-Carb 2500TR liquid scintillation analyzer. Stimulation indices (SI) were calculated by dividing the mean DPM per test group by the mean DPM for the vehicle (VH) control group. EC3 values (concentration of chemical required to induce a 3-fold increase over the VH control) were calculated based on the following equation provided by Ryan and Channey (personal communication, Procter & Gamble).

$$\mathrm{EC3} = 2\hat{}[\log_2 C_A + ((3 - \mathrm{SI_A})/(\mathrm{SI_B} - \mathrm{SI_A})) * (\log_2 C_B - \log_2 C_A)]$$

where C_A = 0.1%, C_B = 0.75%, and SI_A and SI_B are the calculated stimulation indices for 0.1% and 0.75% exposed groups, respectively.

Phenotypic analysis. Lymph node cell phenotypes were analyzed using flow cytometry as described by Manetz and Meade (1999). Mice were exposed to VH (DMF) or increasing concentrations of glutaraldehyde topically on the dorsal surface of each ear (25 μl per ear) for four consecutive days (Table 1). Animals were allowed to rest for 6 days after the final exposure and then euthanized on day 10 by CO_2 inhalation. At the time of euthanization, blood was drawn by cardiac puncture for evaluation of total serum IgE. Draining lymph nodes were collected (two nodes/animal/tube) in 2 ml PBS and were dissociated using the frosted ends of two microscope slides. Cell counts were performed using a Coulter Counter (Z2 model, Beckman Coulter, Miami, FL), and approximately 1×10^6 cells per sample were added to the wells of a 96-well plate. Cells were washed using staining buffer (1% bovine serum albumin/0.1% sodium azide in phosphate buffered saline) and then incubated with Fc block (2.4G2). The cells were then incubated with anti-CD45RA/B220 (RA3-6B2) and anti-IgE antibodies (R-35-72) or the appropriate isotype controls, diluted in staining buffer, washed, and incubated with propidium iodide (PI). All antibodies and isotype controls were purchased from Pharmingen (San Diego, CA). After a final wash, cells were resuspended in staining buffer and analyzed with a Becton Dickinson FACSVantage flow cytometer using a PI viability gate.

Mouse ear swelling test. The mouse ear swelling test (MEST) was performed based on the procedure described by Hayes et al. (1998). Mice, BALB/c or B6C3F1, were exposed topically on the shaved lumbar area with 50 μl of VH, increasing concentrations of glutaraldehyde, or pos-

Table 1
Concentration of chemicals used in the LLNA, phenotypic analysis, and MEST

Chemical	VH	Sensitizing concentration (%)	Challenge concentration[a] (%)
Glutaraldehyde	DMF	0.1, 0.75, 2.5[b]	2.5[b]
HCA	DMF	30	NA
DNFB	acetone	0.15	0.15

[a] Challenge concentration used only in MEST.
[b] Concentrations shown are absolute values.

itive control (DNFB) for 3 days (induction phase). On day 8, before chemical challenge, the thickness of the right ear pinna of each mouse was measured using a modified engineer's micrometer (Mitutoyo Co., Japan). Mice then received a single challenge exposure of 25 μl VH, 2.5% glutaraldehyde, or 0.15% DNFB on the dorsal surface of the right ear pinna (Table 1). Ear measurements were taken 1/2, 24, and 48 h following challenge. Due to the modest response observed at the 24- and 48-h time points, in a subsequent study, a 72-h time point was also evaluated. Using the pre- and post-challenge measurement means, the mean percentage of ear swelling was calculated for each treatment group based on the following equation: [(mean post-challenge ear thickness − mean pre-challenge ear thickness)/ mean pre-challenge thickness] × 100. The mean percentage of ear swelling for each group was compared to the appropriate control for statistical significance.

Total serum IgE. Following euthanization of animals used in the phenotypic analysis and MEST assays, blood samples were collected via cardiac puncture. Sera were collected and total IgE was quantified by ELISA as described by Manetz and Meade (1999) with minor modifications. Briefly, 96-well flat bottom plates (Dynatec Immulon-2) were coated with B1E3 rat anti-mouse IgE and incubated overnight at 4 °C. The B1E3 hybridoma cell line was kindly provided by Dr. Daniel Conrad (MCV/VCU, Richmond, VA). Serum samples, Pharmingen standard (mouse IgE anti-TNP), and internal control (mouse IgE anti-DNP; generously provided by Dr. Daniel Conrad) were serially diluted (1:2) and added to the coated plates. Biotin-conjugated rat anti-mouse IgE (clone R35-92; Pharmingen) was used as the secondary antibody. Streptavidin-alkaline phosphatase (SAP) and *p*-nitrophenyl phosphate were sequentially added to the plates (both obtained from Sigma). Absorbance was determined using a Spectramax Vmax plate reader (Molecular Devices) at 405−605 nm when peak OD values of approximately 1.8 were reached. Data analysis was performed using the IBM Softmax Pro 3.1, and the IgE concentrations for each sample were interpolated from a standard curve using multipoint analysis.

Cytokine analysis. Cytokine analysis was performed using the reverse transcriptase real-time polymerase chain reaction (RT-PCR). The decision to evaluate mRNA vs. protein expression was made because PCR is more sensitive as compared to ELISA, and ConA stimulation is not required to evaluate IL-4 levels. To evaluate the effect of concentration on the sensitization phase, mice (n = 5) were exposed following the dosing protocol for the LLNA. Twenty-four hours after the last exposure, animals were euthanized by CO_2 asphyxiation and the DLNs from individual animals were excised aseptically and placed in RNAlater (Ambion, TX). Total RNA from each animal was isolated following Qiagen's (Valencia, CA) protocol for total RNA isolation from animal tissues and quantitated with a Beckman DU650 mass spectrometer. Two micrograms of RNA per animal was used to synthesize cDNA using Applied Biosystems' TaqMan Reverse Transcription kit (New Jersey, USA) following the manufacturer's instructions in a GeneAmp PCR System 9700 version 1.25 (Applied Biosystems, New Jersey, USA). The real-time PCR reactions were carried out according to manufacturer's instructions using 4 μl cDNA with TaqMan reagents for IL-4, 10, 12, and IFN-γ (Applied Biosystems), and quantified using a Bio-Rad iCycler (Hercules, CA). Data are expressed as fold increase over VH, calculated by the following expression: $2^{-\Delta\Delta Ct}$, where $\Delta\Delta Ct = \Delta Ct(\text{Sample}) - \Delta Ct(\text{VH})$. The $\Delta Ct = Ct(\text{GAPDH}) - Ct(\text{target})$, where Ct = cycle threshold as defined by manufacturer's instructions (Applied Biosystems).

Statistical analysis. Statistical analysis was performed using Graph Pad Prism version 3.0 (San Diego, CA). All data were analyzed by either one-way analysis of variance (ANOVA) when three or more groups were compared or a Student's T test when only two groups were compared. In the ANOVA, when significant differences were detected (P = 0.05), Dunnett's test was used to compare treatment groups with the appropriate control group. Linear regression was used to determine dose responsiveness. Statistical significance is designated by $^{*}P < 0.05$ and $^{**}P < 0.01$.

Results

No signs of systemic toxicity were observed at concentrations up to 2.5% glutaraldehyde

Other than erythema at the site of exposure, all mice appeared clinically normal throughout these studies. There were no exposure-related changes in body weights (data not shown).

A comparable response was obtained in the LLNA using BALB/c as compared to CBA mice

Initial studies compared the sensitizing potential of glutaraldehyde in CBA and BALB/c mice using a standard LLNA. CBA mice were included as they are the recommended strain for use in the LLNA, whereas BALB/c mice are more frequently used for the differentiation of Th1 vs. Th2 responses by cytokine analysis. In CBA mice, glutaraldehyde induced a concentration-dependent increase ($P < 0.01$) in DLN cell proliferation, with counts from the 0.75% and 2.5% exposed animals significantly elevated ($P < 0.01$) over the VH-

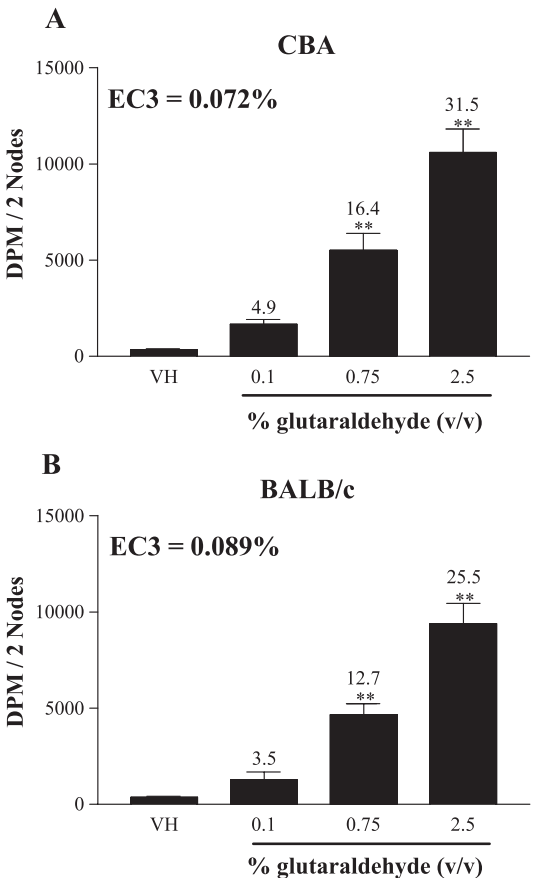

Fig. 1. Analysis of sensitization potential of glutaraldehyde by the LLNA. ^{3}H-thymidine incorporation of DLN cells in (A) CBA mice and (B) BALB/c mice following glutaraldehyde exposure with DMF as the VH control. Data are expressed as group mean DPM (n = 5) ± SE. Numbers appearing above the bars represent the stimulation indices for each concentration tested. EC3 represents the calculated concentration required to produce a SI of 3. **P < 0.01 as compared to VH control.

exposed animals. Exposure to all concentrations tested resulted in stimulation indices of >3, ranging from 4.9 to 31.5, with an EC3 value of 0.072% (Fig. 1A). Studies performed using BALB/c mice gave similar results, with glutaraldehyde inducing a concentration-dependent (P < 0.01) increase in DLN cell proliferation. As with the CBA mice, exposure to 0.75% and 2.5% glutaraldehyde induced significantly elevated (P < 0.01) proliferation as compared to VH exposure. The stimulation indices in BALB/c mice ranged from 3.5 to 25.5, with an EC3 value of 0.089% (Fig. 1B). The positive control group (30% HCA) resulted in a SI of 12.3 in CBA and 8.7 in BALB/c mice. With the exception of the MEST, which was conducted using BALB/c and B6C3F1 mice, all further studies were conducted with BALB/c mice.

Glutaraldehyde exposure induced a concentration-dependent increase in local and systemic IgE levels

To investigate the effect of concentration on the mechanism of sensitization induced by glutaraldehyde exposure, phenotypic analysis was performed to quantify the percentage of B220^{+}- and IgE^{+}B220^{+}-expressing cells in the DLNs. A statistically significant increase (P < 0.01) in absolute and percentage of B220^{+} cells was seen for all exposure groups (Table 2). The B220 test article/VH ratio induced by glutaraldehyde was 2.39, and glutaraldehyde would therefore be identified as a contact sensitizer based on the cut-off ratio of 1.25 proposed by Gerberick et al. (2002). A concentration-dependent increase in the absolute number (P < 0.01) and percentage (P < 0.05) of IgE^{+}B220^{+}-expressing cells was observed following glutaraldehyde exposure reaching statistical significance at 0.75% (P < 0.05) and 2.5% (P < 0.01) exposure levels (Table 2).

Total serum IgE levels from these animals were measured by ELISA. IgE levels were elevated with increasing concentrations of exposure (Table 2), reaching significance in animals exposed to 2.5% glutaraldehyde (1616 ± 302 ng/ml) as compared to VH controls (298 ± 37 ng/ml). The levels of serum IgE in the 0.1% (359 ± 116 ng/ml) and the 0.75% (513 ± 98 ng/ml) exposed groups fell within the historical control range for VH exposed animals in this lab.

Table 2
Phenotypic analysis and total serum IgE results following dermal exposure to increasing concentrations of glutaraldehyde

Groups	Phenotypic analysis				Total IgE (ng/ml)
	B220^{+}		IgE^{+}B220^{+}		
	Absolute no. × 10^{6}	(%)	Absolute no. × 10^{6}	(%)	
DMF	2.5 ± 0.2	(21.5 ± 1.6)	0.3 ± 0.1	(2.2 ± 0.9)	298 ± 37
0.1% Glut	4.6 ± 0.2	(39.7 ± 1.3)**	0.8 ± 0.1	(7.2 ± 0.9)	359 ± 116
0.75% Glut	14.4 ± 0.3**	(47.2 ± 1.1)**	5.8 ± 0.9*	(19.1 ± 2.9)*	513 ± 98
2.5% Glut	23.9 ± 0.3**	(51.5 ± 0.7)**	16.8 ± 1.4**	(36.1 ± 3.1)**	1616 ± 302**

Values present group means (n = 5) ± SE.
*P < 0.05 as compared to VH (DMF) control.
**P < 0.01 as compared to VH (DMF) control.

Exposure to 2.5% glutaraldehyde resulted in an early response, whereas exposure to 0.1% and 0.75% elicited a delayed response in the MEST

Glutaraldehyde was evaluated in the MEST and ear measurements were taken 1/2, 24, and 48 h post challenge. The mice induced and challenged with 2.5% glutaraldehyde exhibited 70.6% ear swelling at 1/2 h post challenge, diminishing to <10% by 24 h, suggesting IgE-mediated immediate hypersensitivity response (Fig. 2A). Conversely, exposure to either 0.1% or 0.75% glutaraldehyde did not yield a significant increase at either the 1/2- or 24-h time points. At 48 h post challenge, a more pronounced and significant increase was observed in ear swelling in mice induced with 0.1% (15.4% swelling) and 0.75% (16.1% swelling) and challenged with 2.5%, as opposed to an 8.3% increase in the group induced and challenged with 2.5% glutaraldehyde (Fig. 2B). For the positive control, induced and challenged with DNFB, a significant increase ($P < 0.01$) in percentage of ear swelling as compared to the background positive control, induced with VH and challenged with DNFB, was observed at both the 24-h (26%) and 48-h (34%) time points. At the 1/2-h time point, a strong irritant effect was seen with no significant increase in the percentage of ear swelling for the 0.15% DNFB-induced/challenged (56 ± 20%) animals as compared to the VH-induced and DNFB-challenged (241 ± 83%) groups. Given the modest response observed in this study, the study was repeated in BALB/c and B6C3F1 and an additional study was conducted in BALB/c mice with an extended time point at 72 h. Similar trends were observed in all studies. These results suggest an immediate (Th2) response in the 2.5% exposed group and a delayed (Th1) response in the 0.1% and 0.75% exposed groups. Furthermore, total serum IgE was significantly elevated ($P < 0.01$) only in the group of animals exposed to 2.5% glutaraldehyde (3942 ± 149 ng/ml) when compared to the VH control (748 ± 95 ng/ml), further supporting a Th2 response in this group.

Exposure to 2.5% glutaraldehyde up-regulated IL-4 mRNA

Cytokine mRNA in the DLNs was analyzed to further evaluate the effect of concentration of exposure on Th1/Th2 balance. Cytokine mRNA levels analyzed included IL-4, -10, -12, and IFN-γ. A concentration-dependent increase ($P < 0.05$) in IL-4 mRNA levels was observed following glutaraldehyde exposure, reaching statistical significance ($P < 0.05$) at the 2.5% exposure concentration (Fig. 3A). Levels of IFN-γ mRNA were not modulated following exposure to glutaraldehyde at this time point (Fig. 3B). Likewise, there

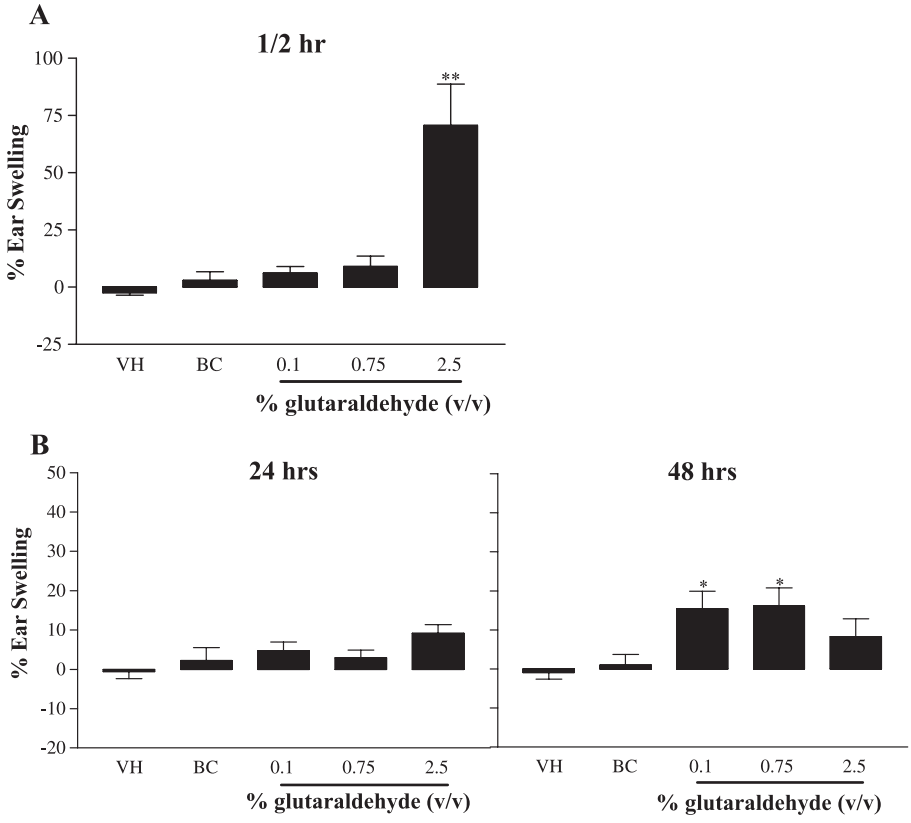

Fig. 2. Percentage of ear swelling following glutaraldehyde exposure at (A) 1/2, and (B) 24 and 48 h post challenge in BALB/c mice. Bars represent mean percentage of ear swelling ($n = 8$) ± SE. *$P < 0.05$ and **$P < 0.01$ as compared to the background control (BC), induced with VH and challenged with 2.5% glutaraldehyde. Data are representative of three studies.

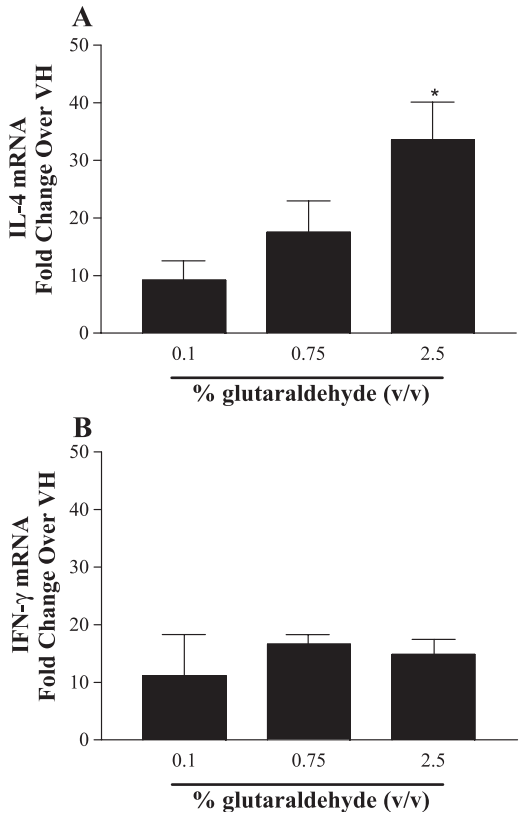

Fig. 3. IL-4 (A) and IFN-γ (B) expression measured by real-time PCR. Results are expressed as fold change over the VH (DMF) control by the $2^{\Delta\Delta Ct}$ method. Bars represent mean ± SE for five animals per group. *P < 0.05 as compared to VH control. Data are representative of two studies.

were no effects on levels of IL-10 or IL-12 mRNA at this time point (data not shown).

Discussion

Despite the fact that glutaraldehyde has long been recognized to cause allergic contact dermatitis (Shaffer and Belsito, 2000), current data suggest that glutaraldehyde may be a significant cause of occupational asthma. According to the Surveillance of Work Related and Occupational Respiratory Disease (SWORD) program in 1992–1997, 4% of all occupational asthma cases reported in the United Kingdom, and 72% of all cases of occupational asthma in nurses were related to glutaraldehyde exposure (McDonald et al., 2000). The mechanism by which glutaraldehyde induces occupational asthma is unclear. In a study of 24 healthcare workers with glutaraldehyde-associated asthma, only 30% had specific IgE antibodies to a glutaraldehyde-modified protein, whereas 7 of 8 workers in the study that underwent specific bronchial provocation test had positive pulmonary responses (Di Stefano et al., 1999). These findings are similar to those reported for TDI-induced asthma where TDI-specific IgE has been demonstrated in only 5–30% of patients (Bernstein, 1996). As LMW compounds can haptenize with many proteins forming newly immunogenic compounds, it can often be difficult to detect these species and the host antibodies (Park et al., 2001). Therefore, the lack of detection of glutaraldehyde or TDI antibodies does not rule out an IgE-mediated response. Data from animal studies have supported the potential for glutaraldehyde to induce both T cell- and IgE-mediated responses. Stern et al. (1989) demonstrated a DTH response in both mice and guinea pigs following topical exposure to glutaraldehyde. Using cytokine fingerprinting, Dearman et al. (1999) demonstrated an elevation of the Th2 cytokines, IL-4 and IL-10, following dermal exposure to 7.5% glutaraldehyde.

The type of hypersensitivity response initiated following chemical exposure is affected by numerous factors which may include chemical structure of the antigen, host genetics, and environmental conditions. Glutaraldehyde is a LMW chemical (100.13 MW) with unsaturated aldehydes that are highly capable of cross-linking proteins (Richards and Knowles, 1968). Because of these properties, glutaraldehyde can form hapten–carrier complexes with host proteins and form multiple immunogenic agents potentially causing divergent responses (Bowes and Cater, 1968; Hopwood et al., 1970).

Atopy, an immune disorder of hypersensitivity, is considered to be an important underlying factor in the development of asthma, rhinitis, and eczema in individuals between the ages of 5 to 25 (Hopkin, 1997; Magnan et al., 2001). Atopic individuals may have higher levels of total IgE and large numbers of eosinophils in their circulation, which may be associated with an inherited genetic mutation in the IL-4 gene. Hershey et al. (1997) identified a point mutation in the IL-4 receptor α protein, resulting in a single amino acid change. The altered IL-4 gene was associated with higher levels of expression of CD23, a regulatory molecule in the production of IgE, than the wild-type alleles. Additionally, studies have shown that IgE production in response to particular allergens is associated with certain HLA class II alleles, indicating that specific MHC–peptide combinations might favor a strong Th2 response (Magnan et al., 2001). For example, mice expressing H-2s and H-2b.d MHC II demonstrated a Th1 vs. Th2 response, respectively, when stimulated with human collagen type IV (Murray et al., 1992). Likewise, a predisposition toward type IV responses may be related to MHC haplotype. Berylliosis is a T cell-mediated pulmonary disease and in exposed workers, the incidence of disease is associated with particular HLA-DP alleles (Fontenot et al., 2000; Saltini et al., 1998).

Although the impact of route, frequency, and amount of exposure in the development of an IgE response has been evaluated (Nelde et al., 2001; Weissman and Lewis, 2002), few studies have investigated the balance between IgE and DTH responses in reference to the above parameters for those chemicals with the potential to induce diverse immune responses. Dose has been proposed as a deciding factor in

the differentiation of Th1/Th2 responses following exposure to pathogens, proteins, and transplantation antigens (Mosmann and Sad, 1996) and the studies presented here suggest that exposure levels may also be an important factor in T cell polarization following chemical exposure. Although the mechanisms underlying these responses have not been fully elucidated, two factors which may be important include antigen density on the antigen presenting cells (APC) and the influence of chemokine and cytokine release from epidermal cells on dendritic cells (DC). Using D011.10 TCR.αβ mice, Hosken et al. (1995) demonstrated the development of a Th2 response at high concentrations of ovalbumin was dependant on endogenous IL-4 production by CD4+ cells. They hypothesized that the normal balance based on the genetic background of the host and immune environment can be overridden by activation of macrophage/monocytes to produce IL-12 driving a Th1 response or immunization with antigen in a manner that induces elevated levels of IL-4 driving a humoral response.

DC are a heterogenous group of APC that have been shown to influence T cell-mediated immunity based on their lineage, maturation stage, and activation signals (Liu et al., 2001; Reis e Sousa, 2001). Effector functions of DC, at least in human cells, have been shown to be influenced by pro-inflammatory cytokines. Therefore, it is possible that aside from the influence of increased antigen loading on the APC, irritating concentrations of glutaraldehyde may induce the release of pro-inflammatory cytokines by epidermal cells which may modulate the Th1/Th2 response through their effects on APC. A related study, using the hapten picryl chloride, demonstrated that skin barrier disruption skewed the immune response toward a Th2 response (Kondo et al., 1998). The induction on INF-γ was down-regulated in these studies in a tape-strip number-dependant fashion. When the response to house dust mite antigen was evaluated, an up-regulation in IL-4 mRNA but not INF-γ was demonstrated. Due to the irritant nature of glutaraldehyde (Ballantyne and Jordan, 2001; Reifenrath et al., 1985), one would expect both an increase in the production of pro-inflammatory cytokines by epidermal cells and a disruption of the barrier integrity of the skin.

In agreement with other published studies (Sailstad et al., 1995), the studies presented here demonstrated a concentration-dependent increase in DLN cell proliferation in the LLNA with an EC3 value of 0.072% in CBA mice as compared to 0.2% reported by Hilton et al. (1998) following glutaraldehyde exposure. Given that the BALB/c mouse is a Th2 prone strain (Nishimura et al., 1997) and therefore possibly more analogous to the individuals with atopy, further studies were conducted in this strain. Whereas the LLNA only evaluates the sensitization phase, the MEST was used in these studies to evaluate the elicitation phase following challenge in previously sensitized mice. Although the delayed response to glutaraldehyde in these studies (16% at 48 h) was not robust, it is in the range of other reported studies. Gad et al. (1986) reported a 24% increase in ear swelling following induction with 1% and challenge with 10% glutaraldehyde using a protocol which included the use of adjuvant and tape stripping in the induction phase.

In the studies presented here, it was demonstrated that exposure to 2.5% glutaraldehyde induced an immediate response, whereas exposure to lower concentrations (0.1 and 0.75%) elicited a modest delayed hypersensitivity response. Supporting these findings, significant elevations in IL-4 mRNA and total serum IgE were only observed in the 2.5% exposed group. In a study by Dearman et al. (1999), exposure to 7.5% glutaraldehyde was shown to induce an elevation in IL-4 and IL-10. The discrepancy in IL-10 induction between the two studies could be due to differences in the evaluation of mRNA vs. protein levels, concentrations (2.5% vs. 7.5%) evaluated, or differences in the exposure regimens used. A concentration-dependent increase in both IL-4 mRNA and $IgE^{+}B220^{+}$ cells was observed in the studies presented here. It is not surprising that increases in IL-4 mRNA and $IgE^{+}B220^{+}$ cells were observed at concentrations lower than those required to induce elevated levels of serum total IgE. IL-4 is required to induce the class switch to IgE, and $IgE^{+}B220^{+}$ cells have been detected before elevated levels of serum IgE (Klink and Meade, 2003). The elevation in $IgE^{+}B220^{+}$ cells relates to local binding of soluble IgE to CD23 on B cells in the draining lymph node which may occur before IgE elevations in the serum. The LLNA is a well-established assay that has been accepted as a stand-alone method for the identification of contact sensitizers (NIEHS, 1999). Although the low concentrations of glutaraldehyde (0.1 and 0.75%) did not induce a robust response in the MEST, they both elicited a positive response in the LLNA with an SI of 4.9 and 16.4, respectively, in the absence of an elevation in IL-4 mRNA, $IgE^{+}B220^{+}$ cells, or total serum IgE.

The animal studies presented here are consistent with the human data demonstrating the potential for glutaraldehyde to produce both contact dermatitis and occupational asthma, and suggest that concentration of chemical exposure may play a pivotal role in the immune response that is elicited.

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