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Differential stimulation of immune function by respiratory and contact chemical allergens

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SUMMARY

The nature of immune responses induced following topical exposure to 2,4-dinitrochlorobenzene (DNCB), a potent contact allergen which lacks the capacity to cause respiratory sensitization, and trimellitic anhydride (TMA), a respiratory allergen with comparatively weak skin-sensitizing potential, have been investigated. Exposure of BALB/c strain mice to concentrations of TMA and DNCB which resulted in equivalent levels of activation (cell proliferation) in lymph nodes draining the site of application (50% TMA and 1% DNCB) induced comparable levels of contact sensitization and IgG anti-hapten antibody production. However, under these conditions, exposure only to TMA resulted in an elevation of serum IgE. Furthermore, while TMA induced IgG2b rather than IgG2a antibody the reverse pattern was observed with DNCB. These data demonstrate that TMA and DNCB elicit qualitatively different immune responses which are consistent with their potential to cause respiratory and contact allergy, respectively. The possibility that the responses induced by these chemicals reflect a differential stimulation of T-helper cell subsets (Th1 and Th2) is discussed.

INTRODUCTION

The ability of many chemicals to cause contact sensitivity is well-known. In addition, it is now clear that exposure to certain chemicals may result in respiratory sensitization.[1] Of interest is the fact that chemicals differ with respect to the type of allergy they may elicit. 2,4-dinitrochlorobenzene (DNCB), for instance, is a potent contact allergen,[2] but fails to cause respiratory allergy in guinea-pigs, and, despite evidence of occupational exposure, appears not to induce symptoms of respiratory sensitization in man.[3] Other chemicals, which may or may not cause allergic contact dermatitis, are able to induce respiratory allergy. Thus, occupational exposure to certain acid anhydrides and aromatic isocyanates may, in some instances, result in respiratory sensitization.[4–7] Furthermore, there is evidence that such chemicals (including, for example, trimellitic anhydride, TMA; p-tolyl isocyanate, TMI and toluene diisocyanate, TDI) may induce pulmonary hypersensitivity in guinea-pigs.[3,8–10]

Contact sensitivity is a delayed-type hypersensitivity reaction. In contrast, chemically induced respiratory allergy is, in many cases, characteristic of an immediate hypersensitivity response. It has been shown that occupational respiratory sensitization to chemical allergens is frequently associated with the presence of specific IgE antibodies,[5–7,11,12] and that guinea-pigs exposed to respiratory sensitizers develop hapten-specific homocytotropic antibody.[3,8,10,13,14] We have recently reported that although both TMA and DNCB are immunogenic in mice, only TMA induces specific IgE following inhalation exposure.[15] It is likely that a unifying characteristic of respiratory chemical allergens is the ability to elicit homocytotropic antibodies.

Compelling evidence exists that there is functional heterogeneity among murine helper T cells and that their secreted products exert potentially reciprocal effects on IgE antibody responses *in vitro*.[16–19] The biological significance of these observations is illustrated by the differential influence of cytokines on IgE production *in vivo*. Thus, the generation and maintenance of IgE responses is dependent upon interleukin-4 (IL-4), a product of Th2 cells,[20,21] while interferon-gamma (IFN-γ), a product of Th1 cells, inhibits IgE antibody formation.[22] In addition to influencing IgE production, Th1 and Th2 cells exert different effects on IgG isotype responses in the mouse.[22,23]

We have speculated previously that the ability of some chemical allergens to induce only contact sensitivity, while others preferentially cause respiratory allergy, reflects differential stimulation of the immune system, and possibly selective activation of Th subsets.[15] In the present study our aim has been to test the hypothesis that contact and respiratory allergens induce qualitatively different immune responses. We have measured the capacity of topically applied TMA and DNCB to stimulate IgE production and to induce hapten-specific IgG1, IgG2a and IgG2b antibodies.

Abbreviations: BSA, bovine serum albumin; DNCB, 2,4-dinitrochlorobenzene; DNFB, 2,4-dinitrofluorobenzene; DNP, dinitrophenol; IFN-γ, interferon-gamma; IL-4, interleukin-4; IL-5, interleukin-5; IL-6, interleukin-6; LNC, lymph node cells; LPS, lipopolysaccharide; NGS, normal goat serum; PBS, phosphate-buffered saline; TMA, trimellitic anhydride; TNBS, 2,4,6-trinitrobenzene sulphonic acid.

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MATERIALS AND METHODS

Mice

Young adult (8–12 weeks old) female BALB/c strain mice (Barriered Animal Breeding Unit, Alderley Park, Cheshire, U.K.) were used throughout these studies.

Chemicals

Trimellitic anhydride (TMA; Aldrich Chemical Co., Gillingham, Dorset, U.K.) and 2,4-dinitrochlorobenzene (DNCB; Sigma Chemical Co., St Louis, MO) were used as commercial preparations dissolved in 4:1 acetone:olive oil (AOO).

Measurement of lymph node cell proliferation

Groups of mice received 25 μl of the relevant concentration of the test chemical on the dorsum of both ears. Five days following exposure all mice were injected intravenously via the tail vein with 250 μl of phosphate-buffered saline (PBS) containing 20 μCi of [^{3}H]methyl thymidine (specific activity 2Ci/mmol; Amersham International, Amersham, Bucks, U.K.). 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 (LNC) was prepared by gentle mechanical disaggregation through a 200-mesh stainless steel gauze. Pooled LNC were washed twice with an excess of phosphate-buffered saline (PBS) and precipitated in 5% trichloroacetic acid (TCA) at 4°. Twelve hours later pellets were resuspended with 1 ml of TCA and transferred to 10 ml of scintillation fluid (Optiphase MP; LKB, Flow, McClean, VA). Incorporation of [^{3}H]thymidine ([^{3}H]TdR) was measured by β-scintillation counting and the results expressed as the mean c.p.m. per node for each experimental group.

Sensitization for, and elicitation of, contact sensitivity

Fifty microlitres of test chemical, or an equal volume of vehicle (AOO) alone, were applied to the shaved flank under an occluded patch. Patches were of lint, covered with latex rubber and secured in place with a Poroplast bandage (Scholl U.K. Ltd, London) and 1 cm tape. The patch was removed after 48 hr and 5 days following sensitization ear thickness was measured using an engineers' micrometer (Moore and Wright, Sheffield, U.K.). Immediately afterwards the dorsum of both ears was treated with 25 μl of the challenge concentration of chemical. Elicitation reactions were measured 24 hr later as the mean percentage increase in ear thickness relative to prechallenge values.

Preparation of hapten–protein conjugates

TMA conjugate. A 40 mol excess of solid TMA was added at hourly intervals for 3 hr to 30 mg/ml bovine serum albumin (BSA; Sigma Chemical Co.) in water. The mixture was stirred at room temperature and the pH maintained at 8 by addition of 1 M sodium hydroxide. The conjugate was desalted on a Sephadex G-25 column (Pharmacia, Uxbridge, U.K.) in PBS, dialysed sequentially against 0·1 M sodium bicarbonate buffer (pH 8), and water, freeze-dried and stored at −20°. The degree of substitution was assessed using a method based upon determination of free amino groups by reaction with 2,4,6-trinitrobenzene sulphonic acid (TNBS).[24] Conjugate and BSA at 1 mg/ml in 0·1 M sodium borate buffer (pH 9·3) were incubated for 20 min at room temperature in the presence of 0·03 M TNBS in borate buffer (pH 9·3). The optical density (OD) at 420 nm was measured. BSA has approximately 30 readily available binding sites per molecule and consequently the degree of substitution (mol/mol) was calculated as follows:

$$\text{substitution} = 1 - \frac{\text{OD sample}}{\text{OD BSA}} \times 30.$$

Dinitrophenol (DNP) conjugate. 2,4-dinitrofluorobenzene (DNFB; Aldrich Chemical Co.), which is antigenically entirely cross-reactive with DNCB but which possesses a higher protein reactivity, was used in the preparation of DNP–conjugates. The methods used were as described for TMA, except that in this case the degree of substitution was determined by absorbance at 360 nm according to the method of Makela & Seppala.[25] Both TMA- and DNP–conjugates used in these studies had substitution ratios of approximately 20:1 (moles hapten:moles protein).

Enzyme-linked immunosorbent assays (ELISA)

Serum IgG antibodies. IgG anti-hapten antibodies and anti-hapten antibodies of subclasses IgG1, IgG2a and IgG2b were detected by ELISA. Plastic microtitre plates (Nunc Immunoplate type II, Nunc, Copenhagen, Denmark) were coated with 100 μg/ml of hapten–protein conjugate in PBS by overnight incubation at 4°. The plates were blocked by incubation for a further 30 min at 37° with 5% normal goat serum (NGS) (I.C.N.) in PBS. Mouse serum samples diluted 1:25 with 0·5% NGS in PBS were added to triplicate wells and incubated for 3 hr at 4°. The plates were then incubated for 3 hr at 4° with peroxidase-labelled goat anti-mouse IgG (Nordic, Tilberg, The Netherlands) diluted 1:8000 with 0·5% NGS in PBS, or for 2 hr at room temperature with peroxidase-labelled sheep anti-mouse IgG1, IgG2a or IgG2b (Serotec, Oxford, U.K.) diluted 1:4000, 1:2000 and 1:4000 in 0·5% NGS in PBS, respectively. Substrate (o-phenylenediamine and urea hydrogen peroxide) was added and the reaction stopped by addition of 0·5 M citric acid after either 5 min (IgG1, IgG2a, IgG2b) or 15 min (IgG). Substrate conversion was measured using an automated reader (Multiskan, Flow Laboratories, Irvine, Ayrshire, U.K.). Between each incubation, plates were washed with PBS containing 0·5% Tween 20. Antibody titres were expressed as mean optical density at 450 nm, corrected for background staining recorded with normal mouse serum at an equivalent concentration.

Total serum IgE. Serum IgE was measured using a sandwich ELISA. Plastic microtitre plates were coated with 2·5 μg/ml rat monoclonal anti-mouse IgE antibody BIE3 (kindly provided by Dr D. H. Conrad, Johns Hopkins University School of Medicine, Baltimore MD) in 0·1 M carbonate buffer (pH 9·6) by overnight incubation at 4°. The plates were then blocked by treatment for 30 min at 37° with 5% NGS in PBS. Test serum samples and a mouse monoclonal IgE anti-DNP (clone SPE-7; ICN Immunobiologicals, Bucks, U.K.) diluted to various extents in 0·5% NGS in PBS were added to triplicate wells and the plates incubated for 3 hr at 4°. Plates were then incubated at 4° for 2 hr with 100 ng/ml biotin-labelled rat anti-mouse IgE (Serotec) and for a further 50 min at room temperature with a 1:1000 dilution of streptavidin–horseradish peroxidase conjugate (Serotec). Substrate (as detailed above) was added and the reaction terminated after 15 min by addition of 0·5 M citric acid. Between each incubation the plates were washed with PBS containing 0·5% Tween 20. Optical density at 450 nm was measured as described above. The concentration of IgE in test

Table 1. Induction of LNC proliferation by TMA and DNCB

TMA		DNCB	
% (w/v)	[^{3}H]TdR incorporation mean c.p.m./node $\times 10^{-2}$	% (w/v)	[^{3}H]TdR incorporation mean c.p.m./node $\times 10^{-2}$
0	0·39	0	0·26
1	0·45	0·05	0·34
2·5	0·96	0·1	0·70
5	1·27	0·25	2·55
10	2·55	0·5	4·77
25	4·75	1	6·45
50	7·23	2	10·69

Groups of mice ($n=4$) received 25 μl of various concentrations of either TMA or DNCB on the dorsum of both ears. Control mice received an equal volume of vehicle (AOO) alone. Five days later all mice received 250 μl of PBS containing 20 μCi of [^{3}H]methyl thymidine i.v. via the tail vein. Mice were killed 5 hr later and draining auricular lymph nodes isolated and pooled for each experimental group. Results are expressed as the mean c.p.m./node.

Table 2. Contact sensitization by TMA and DNCB

Chemical	Sensitization (w/v)	Challenge (w/v)	Mean % increase in ear thickness ± SE
TMA	50%	25%	16·7 ± 4·6
	0·95%	25%	1·8 ± 0·8
	0	25%	0·6 ± 0·2
DNCB	1%	0·75%	21·2 ± 3·9
	0	0·75%	3·5 ± 0·9

Groups of mice ($n=8$) received 50 μl of either TMA (50% or 0·95%) or DNCB (1%) under an occluded patch. Control mice received an equal volume of vehicle (AOO) alone under the same conditions. Patches were removed after 48 hr. Five days following sensitization the ear thickness of all mice was measured immediately prior to challenge of the dorsum of both ears with 25 μl of a subinflammatory concentration of the test chemical (25% TMA, 0·75% DNCB). Ear thickness was re-evaluated 24 hr later and elicitation reactions recorded as the mean percentage increase in ear thickness relative to prechallenge values.

serum samples was derived from a standard curve for monoclonal mouse IgE. Results are expressed as serum IgE concentration (μg/ml).

RESULTS

LNC proliferation

Our objective was to compare the capacity of TMA and DNCB to stimulate IgE production and to induce IgG anti-hapten antibody under conditions of approximately equivalent immunogenicity. Therefore, rather than use equimolar test concentrations, we elected to examine responses to these chemicals using concentrations which, when applied topically, elicited equivalent levels of immune activation in draining lymph nodes. A representative experiment, in which immune activation following a single exposure to various concentrations of TMA or DNCB has been measured as a function of *in situ* LNC proliferation, is detailed in Table 1. The data demonstrate that at equivalent concentrations DNCB induced a markedly greater proliferative response by draining LNC. On the basis of this series of experiments, we chose in future studies to compare responses induced by 1% DNCB and 50% TMA.

Contact sensitization

We have previously suggested that the vigour of the T-lymphocyte proliferative response in the lymph node(s) draining the site of primary exposure to a contact allergen directly influences the degree of sensitization achieved.[26] In accord with this we observed that exposure of mice to concentrations of TMA and DNCB which induced approximately equivalent levels of LNC proliferation (50% and 1%, respectively) resulted in similar levels of contact sensitization (Table 2). The data contained in Table 2 also serve to demonstrate that DNCB is a considerably more potent contact allergen than TMA. Following exposure to equimolar (0·49 mM) concentrations of DNCB or TMA (1% DNCB or 0·95% TMA) only DNCB induced contact sensitization (Table 2).

IgG anti-hapten antibody

Groups of mice ($n=5$) received various concentrations of either TMA or DNCB bilaterally on the shaved flanks. Seven days later mice received a second application of the same chemical on the dorsum of both ears. Mice were exsanguinated 8, 14 and 21 days following the commencement of exposure and individual sera examined for the presence of IgG anti-hapten antibody. Results are summarized in Fig. 1. Topical exposure to concentrations of TMA of 5% or more induced an IgG anti-TMA response which was maximal by Day 14. No such response was observed following exposure to 1% TMA. Treatment with either 0·5% or 1% DNCB also induced an IgG anti-hapten (anti-DNP) antibody response which was maximal 2 weeks following exposure. Initial treatment with lower concentrations of DNCB (0·05% or 0·1%) did not provoke an IgG response (Fig. 1). Thus, topical exposure to concentrations of TMA and DNCB (50% and 1%, respectively) which result in approximately equivalent levels of LNC proliferation and contact sensitization, also induce the production of specific IgG antibody.

Serum IgE

We have observed previously that inhalation exposure to TMA, but not to DNCB, results in the appearance of serum IgE anti-hapten antibody.[15] We now report that topical exposure to TMA causes an increase in the serum concentration of IgE. In preliminary studies it was found that the concentration of IgE in normal BALB/c mouse serum varied between 0·1 and 0·3 μg/ml (values broadly similar to those reported previously by Azuma *et al.*[27] who recorded IgE serum concentrations in untreated BALB/c mice of between <20 ng/ml to over 600 ng/ml).

Mice received 50 μl of 50% TMA on both shaved flanks and

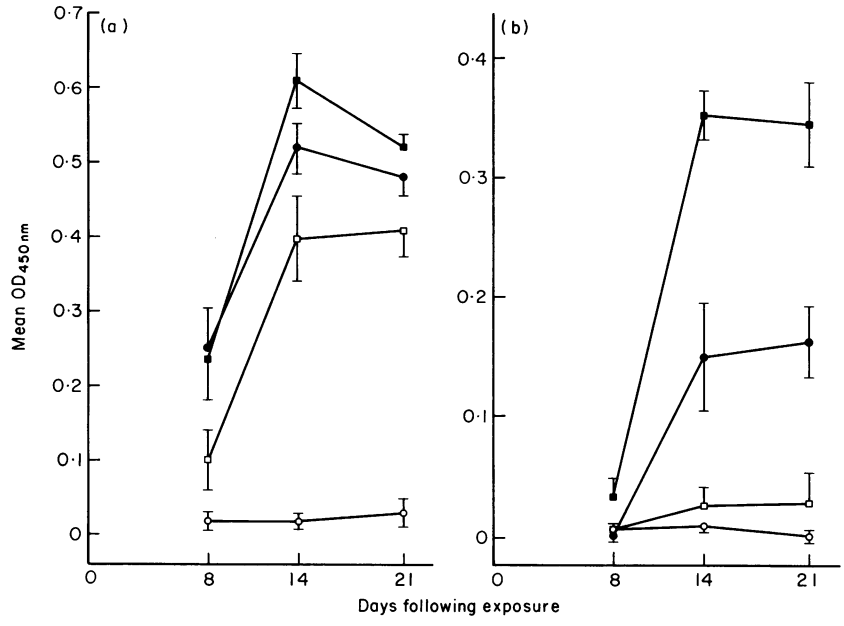

Figure 1. IgG anti-hapten antibody following topical administration of TMA and DNCB. Groups of mice ($n = 5$) were exposed to either TMA (a) or DNCB (b). Mice received 50 μl of test chemical bilaterally on the shaved flanks [TMA: 1% (○), 5% (□), 10% (●) or 50% (■); DNCB: 0·05% (○), 0·1% (□), 0·5% (●) or 1% (■)]. Seven days later mice received 25 μl of a 1:1 dilution of the same solution on the dorsum of both ears. At various periods following the commencement of exposure groups of mice were exsanguinated and individual sera tested for IgG anti-hapten antibody by ELISA. Mean values ± SE are illustrated.

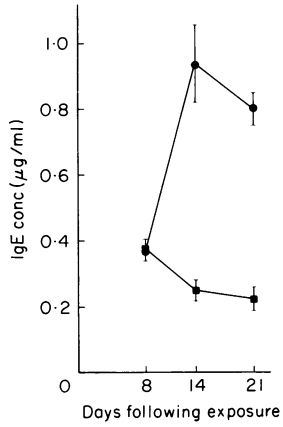

Figure 2. Serum IgE following topical administration of TMA and DNCB. Groups of mice were exposed to either 50% TMA (●) or 1% DNCB (■). Mice received 50 μl of the test chemical on both shaved flanks. Seven days later all animals received 25 μl of the same chemical (25% TMA or 0·5% DNCB) on the dorsum of both ears. Groups of mice ($n = 10$) were exsanguinated, and IgE in individual sera measured by ELISA, 8, 14 and 21 days following the commencement of exposure. Mean values ± SE are illustrated.

7 days later 25 μl of 25% TMA on the dorsum of both ears. Two weeks following the first exposure the mean concentrations of serum IgE had risen to more than 0·9 μg/ml (Fig. 2). In contrast, exposure to 1% DNCB under the same conditions failed to induce a similar elevation of serum IgE.

Isotype distribution of IgG anti-hapten antibodies

The relative amounts of IgG1, IgG2a and IgG2b anti-hapten antibody were measured using isotype-specific ELISA at various periods following exposure to either TMA or DNCB. Groups of mice ($n = 10$ or, in one case, $n = 9$) received 50 μl of test chemical on each shaved flank, and 25 μl of the same chemical on the dorsum of both ears 7 days later. Mice were exsanguinated 8, 14 and 21 days after the initiation of exposure. The results obtained with individual serum samples are illustrated in Figs 3 and 4.

At every time-point examined all mice exposed to TMA exhibited significant levels of IgG1 anti-TMA antibody. The interesting observation was, however, that, at all periods, serum from TMA-treated mice invariably contained substantially more IgG2b than IgG2a anti-hapten antibody. On the basis of OD_{450nm} readings the IgG2a:IgG2b anti-TMA antibody ratios ranged from 1:3·0 to 1:9·9 (mean 1:6·3) at 8 days, from 1:4·7 to 1:8·5 (mean 1:7·2) at 14 days, and from 1:3·8 to 1:5·9 (mean 1:5·1) at 21 days (Fig. 3).

In common with the data recorded in Fig. 1, exposure to DNCB in most cases failed to induce detectable levels of IgG anti-hapten antibody at 8 days, although by 14 and 21 days following treatment all mice exhibited a significant response. At these time-points sera from all mice contained IgG1 anti-DNP. However, in sharp contrast to TMA, DNCB elicited a much great IgG2a than IgG2b anti-hapten response. IgG2a:IgG2b anti-DNP ratios varied from 1:0·05 to 1:0·23 (mean 1:0·15) at 14 days, and from 1:0·02 to 1:0·63 (mean 1:0·16) at 21 days (Fig. 4).

Although the use of different hapten–protein conjugates for measurement of antibodies of different specificity prohibits

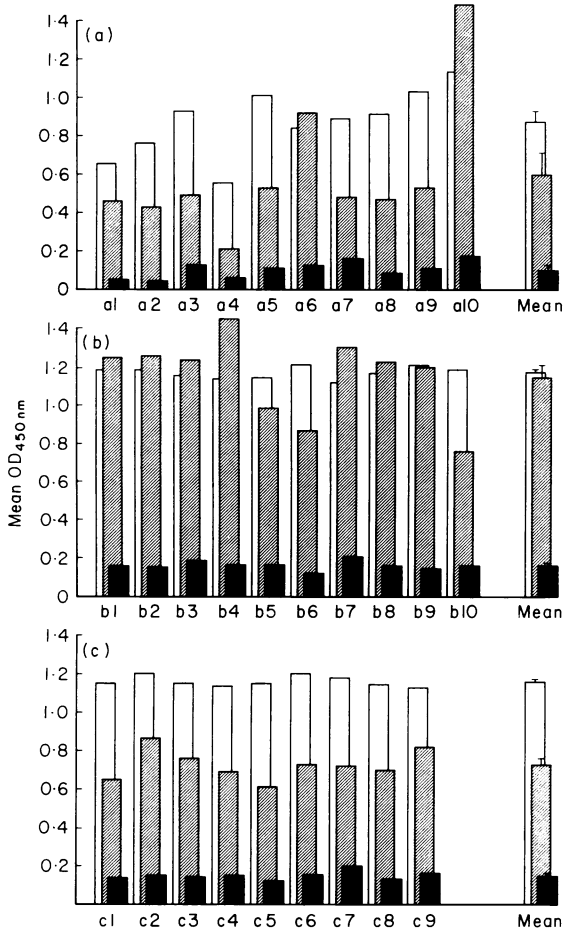

Figure 3. IgG isotype anti-hapten responses following topical administration of TMA. Groups of mice ($n=9$ or 10) received 50 μl of 50% TMA on both shaved flanks. Seven days later all mice received 25 μl of the same chemical (25%) on the dorsum of both ears. Mice were exsanguinated 8 (a), 14 (b) or 21 (c) days following the initiation of exposure. The presence of IgG1 (□), IgG2a (■) and IgG2b (▩) anti-TMA antibody was measured by isotype-specific ELISA. Results obtained with sera from individual mice, together with mean values ± SE, are illustrated.

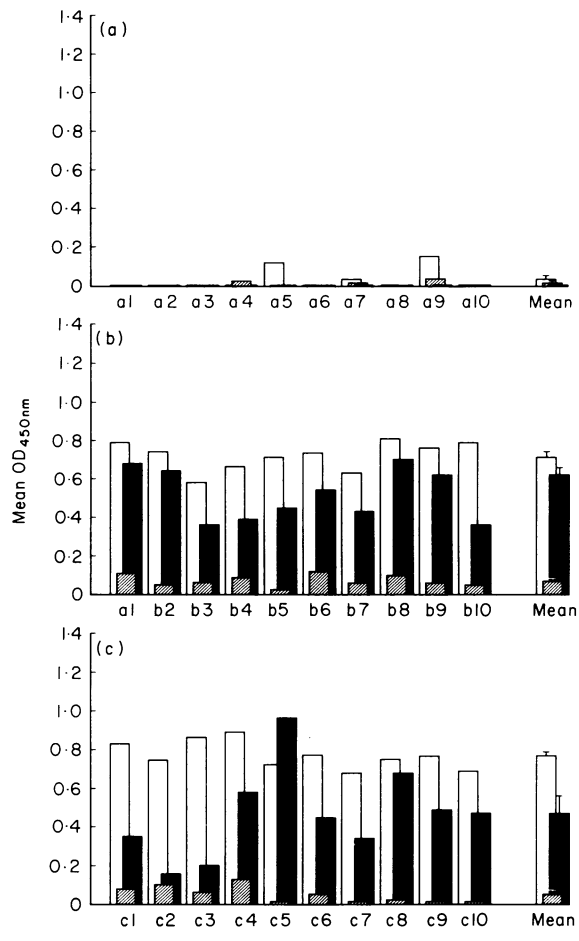

Figure 4. IgG isotype anti-hapten responses following topical administration of DNCB. Groups of mice ($n=10$) received 50 μl of 1% DNCB on both shaved flanks. Seven days later all mice received 25 μl of the same chemical (0·5%) on the dorsum of both ears. Mice were exsanguinated 8 (a), 14 (b) or 21 (c) days following the initiation of exposure. The presence of IgG1 (□), IgG2a (■) and IgG2b (▩) anti-DNP antibody was measured by isotype-specific ELISA. Results obtained with sera from individual mice, together with mean values ± SE are illustrated.

direct quantitative comparisons of the absolute concentration of IgG2a and IgG2b anti-hapten antibody present in each case, it is clear that TMA and DNCB differ markedly with respect to the sub-type of IgG2 which is preferentially induced.

DISCUSSION

Evidence is presented that under conditions of exposure where TMA and DNCB elicit comparable lymph node cell proliferation, contact sensitization and total IgG anti-hapten antibody responses, there are marked differences in stimulation of IgE and in the isotype distribution of IgG antibodies. Although both TMA and DNCB induce IgG1 anti-hapten antibody, TMA preferentially elicits IgG2b, and DNCB IgG2a, antibodies.

We have reported previously that inhalation exposure of mice to TMA, but not to DNCB, results in the appearance of serum IgE antibody.[15] In this study topical exposure only to TMA caused an increase in total serum IgE (coincident with the appearance of IgE anti-TMA antibody; data not presented). The stimulation of IgE by TMA is compatible with the ability of this chemical to cause respiratory sensitization in both man and guinea-pigs. Likewise, the fact that DNCB is a potent contact allergen, but appears not to induce respiratory sensitization, is consistent with its failure to provoke IgE production. The inability of DNCB immunization to induce IgE antibody is not, however, attributable to the nature of the hapten (DNP) *per se*. IgE anti-DNP antibody may be provoked in mice by pretreatment with 2,4-dinitrophenol-*Bordetella pertussis* and subsequent challenge with DNP-ovalbumin.[28]

It is relevant to consider the differential responses which result from exposure to TMA and DNCB in the context of information available regarding the role of helper T-cell populations, and their soluble products, in the regulation of immunoglobulin switching and IgE production. IL-4 has been shown to

stimulate IgE and IgG1 production by lipopolysaccharide (LPS)-activated B lymphocytes *in vitro*,[17,29-32] and to be necessary for the initiation and maintenance of IgE responses *in vivo*.[21] In contrast IFN-γ inhibits IgE production and stimulates IgG2a secretion.[17,22] IL-4 is a product of Th2 cells and IFN-γ of Th1 cells,[16] and it has recently been shown that clones of Th1 and Th2 cells specific for epitopes on the same antigen induce antibody of different isotypes.[23] It is intriguing that, inasmuch as DNCB induces IgG2a antibody (but not IgE) and TMA stimulates IgE production, the results observed with these chemicals are broadly compatible with a differential activation of Th subsets. Compatible also is the fact that Th2 cells appear not to provoke IgG2a antibody.[23] One apparent anomaly is, however, the evidence that IL-4 inhibits IgG2b production by LPS-stimulated B cells,[33] whereas, in the experiments reported here, TMA exposure resulted in a comparatively marked IgG2b anti-hapten response. It may, however, be unwise to equate too closely the biological effects of IL-4 *in vitro* with the possible influence of Th2 activation *in vivo*. Th2 clones, in addition to IL-4 (and a variety of other cytokines), produce interleukin-5 (IL-5).[34] There is evidence that IL-5 acts synergistically with IL-4 in the enhancement of IgG1 and IgE production by LPS-activated B cells *in vitro*.[18,35] IL-5 does not appear to inhibit IgG2b production and may in fact cause a modest elevation in the concentration of this isotype in the supernatants of LPS-driven B-cell cultures.[18] There is, however, no evidence to suggest that IL-5 is able to modify the IL-4-mediated inhibition of IgG2b.[18] Interestingly it has been claimed that interleukin-6 (IL-6), which is also a product of Th2, but not of Th1 cells,[19] may act as a switch-inducing factor for IgG2b (and IgG1).[36] It is not unreasonable to speculate that, despite evidence for inhibition of IgG2b by IL-4, the cytokine products of Th2 cells acting in concert, may (according to their relative concentration and availability) exert different, and possibly opposite, effects *in vivo*.

If TMA and DNCB selectively (but not exclusively) activate Th2 and Th1 cells, respectively, then one is forced to consider how this might occur. There is evidence that, in addition to the spectrum of cytokines they secrete, Th1 and Th2 cells differ in other ways, including their relative radiosensitivity,[37] requirements for tolerance induction[38] and expression of membrance Fc receptors.[39] It is relevant that Th1 and Th2 appear also to differ with respect to the co-stimulatory signals required for activation[40] and the pathway for signal transduction following activation.[41] Importantly, recent studies by Murray *et al.*[42] indicate that the MHC class II haplotype also influences T-cell subset activation, determining whether, in response to a particular antigen, IFN-γ- or IL-4-producing cells become activated. It may be that the responses observed following exposure to TMA and DNCB arise from differential antigen processing and/or presentation resulting in selective T-cell activation.

Although formal proof that DNCB and TMA elicit responses by different functional subpopulations of T lymphocytes will require further experimentation, such would provide an attractive explanation of the different biological consequences resulting from exposure to respiratory and contact allergens. Th1 cells are inflammatory. Th1, but not Th2 cells, transfer delayed-type hypersensitivity (DTH) responses to naive mice;[43] indeed it has been found that IFN-γ alone can reverse the inhibition of DTH caused by treatment of mice with anti-CD4 monoclonal antibodies.[44] A predominantly Th1 response would also facilitate macrophage activation through release of IFN-γ, lymphotoxin and granulocyte–macrophage colony-stimulating factor (GM-CSF), the first two of these cytokines being produced only by Th1 cells.[19] Selective Th2 activation would result in a strong antibody response including the production of IgE. In addition to IgE, several features of immediate-type hypersensitivity responses would be augmented by Th2-derived cytokines. IL-5 is a potent stimulator for the growth and differentiation of eosinophils,[45] and IL-4 is a co-stimulator for growth of mast cells.[46] Following exposure to chemical allergens the type of hypersensitivity preferentially induced would therefore be dependent upon the balance between Th1 and Th2 activity; a balance that would presumably be determined by the nature and persistence of the immunogenic signal, and influenced further by endogenous mechanisms through which Th1 and Th2 cells may exert mutually antagonistic effects. A newly defined product of Th2 cells (cytokine synthesis inhibitory factor, CSIF, or interleukin-10) inhibits cytokine production by, and therefore the effector function of, Th1 cells,[47,48] while IFN-γ selectively suppresses the proliferation of Th2 clones.[49] A preferential, rather than absolute, activation of Th2 cells by TMA, resulting in a balance between Th1- and Th2-derived effector molecules, would accommodate the fact that, as illustrated by the present study, this chemical has a definite, albeit relatively weak, ability to induce contact hypersensitivity.

The exact mechanisms whereby TMA and DNCB stimulate qualitatively different immune responses, and the extent to which the activities of these chemicals serve as a paradigm for respiratory and contact allergens in general, awaits further clarification. Nevertheless it is possible to conclude that, in addition to IgE stimulation, TMA and DNCB exhibit marked differences in the isotype distribution of induced IgG antibodies. Extension of these studies to other chemical allergens will determine whether isotype patterns provide a reliable correlate of the form chemically induced hypersensitivity reactions might take.

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