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Contribution of $CD4^+$ and $CD8^+$ T lymphocyte subsets to the cytokine secretion patterns induced in mice during sensitization to contact and respiratory chemical allergens

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SUMMARY

Chemical allergens of different types, those that cause in humans allergic contact dermatitis or occupational asthma, induce in mice divergent immune responses characteristic, respectively, of T-helper 1 (Th1)- and Th2-type cell activation. Such responses are associated with the development of different cytokine secretion patterns by draining lymph node cells (LNC), such that contact allergens stimulate vigorous interferon-γ (IFN-γ) production, but little secretion of the Th2 cytokines interleukin-4 and interleukin-10 (IL-4 and IL-10), whereas the converse pattern is provoked by respiratory allergens. Using selective depletion with antibody and complement we have here examined the relative contribution of $CD4^+$ and $CD8^+$ T lymphocytes to the cytokine secretion patterns of draining LNC isolated from mice sensitized to chemical allergens. Mice received repeated topical applications of respiratory allergens, trimellitic anhydride (TMA) or diphenylmethane diisocyanate (MDI), or of contact allergens 2,4-dinitrochlorobenzene (DNCB) or formaldehyde. Thirteen days following the initiation of exposure the production by draining LNC of IL-10, IFN-γ and mitogen (concanavalin A)-inducible IL-4 was measured by enzyme-linked immunosorbent assay (ELISA) after various periods of culture. It was found that the high levels of IL-4 and IL-10 secretion stimulated by TMA or MDI, and the lower levels of these cytokines induced by DNCB or formaldehyde, were in all cases dependent upon the presence of $CD4^+$ cells. In contrast, the comparatively high concentrations of IFN-γ observed following exposure to contact allergens were found to be derived from $CD4^+$ cells, and in the case of DNCB from $CD8^+$ cells also. The low levels of IFN-γ induced by treatment with TMA or MDI were associated largely or wholly with $CD8^+$ cells. These data indicate that the type 2 cytokine responses induced to different extents by both contact and respiratory chemical allergens are almost exclusively a function of $CD4^+$ cells, but that IFN-γ is produced by either $CD4^+$ and $CD8^+$ cells in the case of contact allergens or largely by $CD8^+$ cells in the case of chemical respiratory allergens.

INTRODUCTION

The characteristics of immune responses are to an important extent governed by the activity of T-helper (Th) cell subpopulations and their cytokine products. In the mature immune response two phenotypes, designated Th1 and Th2, predominate and are distinguished as a function of the cytokines they secrete.[1,2] While these populations have in common the ability to produce some cytokines, only Th1 cells secrete interferon-γ (IFN-γ), interleukin (IL)-2 and tumour necrosis factor-β and only Th2 cells secrete IL-4, IL-5, IL-6 and, in mice, IL-10.[2,3] Such heterogeneity may play a decisive role in the development of allergic diseases. Cytokine products of Th2 cells favour the induction of acute allergic responses and immediate-type hypersensitivity, with IL-4 and IL-5 promoting, respectively, the production of immunoglobulin E (IgE) antibody and the development, localization and function of eosinophils.[4–6] Conversely, Th1 cells and their soluble product, IFN-γ, inhibit the stimulation of IgE antibody responses and the elicitation of immediate-type allergic reactions, favouring instead the development of cell-mediated immunity and delayed hypersensitivity.[7–10]

We have shown previously that chemical allergens of different types induce divergent immune responses characteristic of selective Th cell subset activation in BALB/c strain mice.[11–14] Such responses to contact and respiratory chemical allergens are associated, respectively, with the emergence of Th1- and Th2-type cytokine production patterns.[15–17] Thus, draining lymph node

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Abbreviations: AOO, 4:1 acetone:olive oil; DNCB, 2,4-dinitrochlorobenzene; ELISA, enzyme-linked immunosorbent assay; IFN-γ, interferon-γ; IL, interleukin; LNC, lymph node cells; MDI, diphenylmethane diisocyanate; Th, T helper; TMA, trimellitic anhydride.

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cells (LNC) isolated from mice treated repeatedly on the skin with contact sensitizing chemicals express high levels of IFN-γ, but only low or undetectable levels of IL-4 and IL-10. Under the same conditions of exposure, treatment of mice with chemicals known in humans to cause sensitization of the respiratory tract and occupational asthma, results in the converse pattern of cytokine secretion.[16,17] It is not necessarily the case, however, that such cytokines derive exclusively from CD4⁺ Th cells. There is a realization now that a similar functional heterogeneity exists among CD8⁺ T lymphocytes, and two populations, Tc1 and Tc2, have been described which display cytokine selectivity comparable, respectively, with Th1 and Th2 cells.[18–21] There are indications that functional subsets of CD8⁺ lymphocytes, and in particular Tc1-type cells, may play important roles as regulators or effectors of allergic reactions in experimental animals.[22–24]

In the present investigation we have therefore questioned the extent to which CD8⁺ T lymphocytes contribute toward the characteristic cytokine secretion patterns that develop in mice exposed to chemical allergens. Mice of BALB/c strain were exposed topically to chemicals known to cause respiratory sensitization in humans, trimellitic anhydride (TMA) or diphenylmethane diisocyanate (MDI)[25,26] or to contact allergens known or suspected not to induce sensitization of the respiratory tract, 2,4-dinitrochlorobenzene (DNCB) or formaldehyde.[27,28] The production of IFN-γ, IL-10 and mitogen-inducible IL-4 by draining LNC or by LNC selectively depleted of CD4⁺ or CD8⁺ T lymphocytes was measured by enzyme-linked immunosorbent assays (ELISA).

MATERIALS AND METHODS

Mice

Young adult (8–12 weeks old) female BALB/c strain mice (Harlan Olac, Bicester, UK) were used throughout these studies.

Chemicals

Formalin (37% formaldehyde) and DNCB were obtained from Sigma Chemical Co., St. Louis, MO and TMA from Aldrich Chemical Co., Gillingham, UK. MDI was provided by ICI Polyurethanes (Everberg, Belgium). Chemicals were used as commercial preparations and, with the exception of formaldehyde, dissolved in 4 : 1 acetone:olive oil (AOO). Formalin was dissolved in acetone.

Sensitization of mice

Groups of mice ($n = 20$) received 50 μl of chemical (10% TMA, 1% DNCB, 1% MDI or 50% formaldehyde) bilaterally on each shaved flank. Five days later this treatment was repeated. After a further 5 days, 25 μl of chemical was applied to the dorsum of both ears daily for 3 consecutive days.

Preparation of draining lymph node cells

Thirteen days after the initiation of exposure, draining auricular lymph nodes were excised and pooled for each experimental group. A single cell suspension of LNC 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 LNC suspended at 10^7 cells/ml in RPMI-1640 growth medium (Gibco, Renfrewshire, UK) supplemented with 25 mM HEPES, 400 μg/ml streptomycin, 400 μg/ml ampicillin and 10% heat-inactivated fetal calf serum (RPMI-FCS).

Complement depletion of CD4⁺ and CD8⁺ T cells from LNC

Lymph node cells prepared as described above were incubated at 2×10^7 cells/ml at 4° for 45 min with 4 μg/ml rat monoclonal anti-L3T4 (anti-mouse CD4; clone YTS 191·1.2) or anti-Lyt.2 (anti-mouse CD8; clone YTS 169·4) diluted in RPMI-FCS. Both antibodies were of IgG2b isotype and were obtained from Harlan SeraLab, Crawley Down, UK. Control preparations were incubated with the same concentration of rat IgG2b myeloma protein (Serotec, Kidlington, UK) diluted in RPMI-FCS. Lymphocyte suspensions were washed once and suspended in RPMI-FCS supplemented with 10% low toxicity rabbit complement (Harlan SeraLab) and incubated for a further 45 min at 37° in a humidified atmosphere of 5% CO_2 in air. Cells were washed twice and viable cell counts performed by exclusion of 0·5% trypan blue. Cells were resuspended in RPMI-FCS at 10^7 cells/ml.

Culture of LNC

Unfractionated LNC, LNC treated with isotype-matched control immunoglobulin and LNC depleted of CD4 and CD8 T lymphocytes were seeded into 24-well tissue culture plates at 10^7 cells/ml and maintained at 37° in a humidified atmosphere of 5% CO_2 in air. Culture was performed in the presence or absence of 2 μg/ml concanavalin A (con A; Sigma). Culture was terminated after 12–120 hr, supernatants collected, centrifuged at 100 *g* for 5 min and stored at −70° prior to cytokine analysis.

Cytokine analyses

Interleukin-4. The IL-4 content of culture supernatants was measured by sandwich enzyme-linked immunosorbent assay (ELISA) as described previously.[15] Plastic microtitre plates (Nunc, Copenhagen, Denmark) were coated by overnight incubation at 4° with 2·5 μg/ml rat monoclonal anti-mouse IL-4 antibody (Genzyme, Cambridge, MA) in 0·1 M carbonate buffer (pH 9·6). The plates were blocked for 30 min at 37° with 5% FCS in phosphate-buffered saline (PBS; pH7·2). Recombinant murine IL-4 ($1–2 \times 10^7$ 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 hr at room temperature. Plates were then incubated for 2 hr at room temperature with 8 μg/ml of goat anti-mouse IL-4 (R & D Systems Europe, Abingdon, UK) and for a further 2 hr at room temperature with a 1 : 500 dilution in RPMI-FCS 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 addition of 0·5 M 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, 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 300 pg/ml. Samples were analysed in triplicate, standard errors were <10% in most experiments.

Interferon-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° with 0·5 μg/ml hamster monoclonal anti-mouse IFN-γ antibody (Genzyme) in 0·1 M carbonate buffer (pH 9·6). The plates were blocked for 30 min at 37° with 5% FCS in phosphate buffered saline (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 hr at room temperature. The plates were then incubated for 2 hr at room temperature with 4 μg/ml goat anti-mouse IFN-γ (Genzyme) diluted in RPMI-FCS and for a further 2 hr 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 M 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). 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 errors were <10% in most experiments.

Interleukin-10. The IL-10 content of culture supernatants was analysed using a dual-monoclonal sandwich ELISA. Plastic microtitre plates were coated with 2 μg/ml rat monoclonal anti-mouse IL-10 antibody (Pharmingen, San Diego, CA) in 0·1 M carbonate buffer (pH 9·6) by overnight incubation at 4°. The plates were then blocked by treatment for 2 hr 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 medium diluted to various extents in RPMI-FCS were added to triplicate wells and the plates incubated for 4 hr at room temperature. Plates were then incubated for 45 min at room temperature with 2 μg/ml biotinylated rat anti-mouse IL-10 (Pharmingen) and for a further 30 min at room temperature with 2·5 μg/ml ExtrAvidin peroxidase (Sigma) diluted in 5% FCS in PBS. Enzyme substrate (tetramethyl benzidine and hydrogen peroxide) was added and the reaction terminated after 10 min by the addition of 2 M sulphuric acid. Between each incubation, the plates were washed as above. Optical density at 450 nm was measured. 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 errors were <10% in most experiments.

RESULTS

Cytokine secretion profiles by unfractionated LNC prepared from mice sensitized with TMA or DNCB

On the basis of flow cytometric analyses, untreated LNC prepared from the draining nodes of TMA- or DNCB-sensitized mice contained ≈35% $CD4^+$ cells and 15% $CD8^+$ cells. Similar values were obtained with LNC treated with the isotype-control preparation of rat IgG2b myeloma protein (data not presented). The production by these populations of IL-10, IFN-γ and con A-inducible IL-4 during various periods of culture was measured using cytokine-specific ELISAs. Consistent with previous investigations,[16] repeated exposure of mice to TMA was found to result in the vigorous production by LNC of mitogen-inducible IL-4 that was maximal after 12–24 hr culture. Treatment of control LNC from naive unsensitized mice with con A failed to stimulate the production of IL-4 (data not presented), an observation again consistent with the results of previous studies.[15,16] Sensitization with TMA also resulted in the spontaneous production by draining LNC of IL-10; in these experiments maximal production of this cytokine being recorded following culture of cells for 120 hr. Despite the production of IL-10 and mitogen-inducible IL-4, LNC prepared from TMA-sensitized mice secreted only very low concentrations of IFN-γ (Fig. 1). Treatment of LNC from TMA-sensitized mice with IgG2b myeloma protein and complement had no influence on this pattern of cytokine secretion (Fig. 1). A very different profile of cytokine secretion was observed with draining lymph node cells isolated from mice sensitized with DNCB. In this case only low or undetectable levels of IL-4 and IL-10 were produced. However, LNC from DNCB-sensitized mice secreted high concentrations of IFN-γ; peak production being recorded after culture for 120 hr (Fig. 1). Here again, the isotype-control preparation of rat IgG2b myeloma protein had no affect on cytokine secretion patterns (Fig. 1). As no differences were observed between untreated LNC and those treated with IgG2b myeloma protein, in subsequent experiments only the latter control group was used.

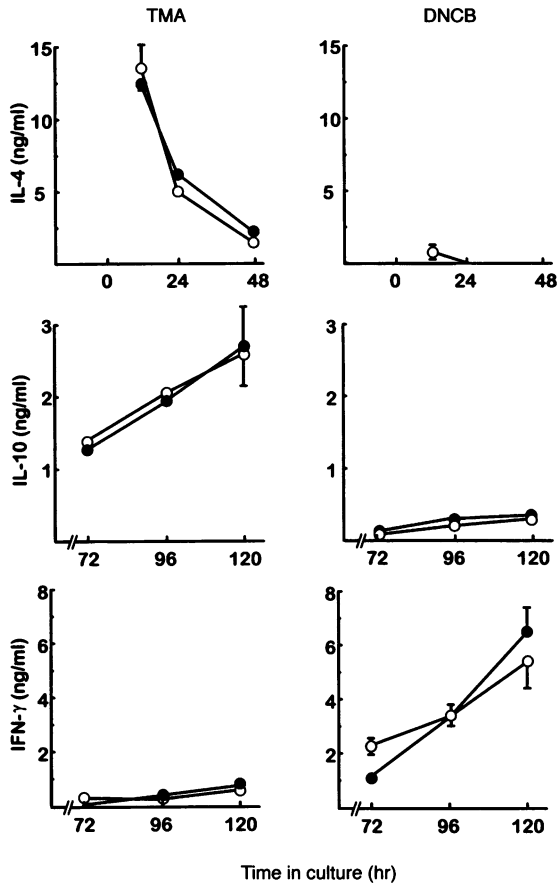

Figure 1. Cytokine secretion profiles by unfractionated LNC prepared from mice sensitized with TMA or DNCB. LNC were isolated from mice following chronic topical exposure to 10% TMA or 1% DNCB. Cells were untreated (●) or cultured with an isotype control preparation of IgG2b rat myeloma protein and complement (○). Cytokine concentrations were measured by ELISA in supernatants prepared following culture of LNC for various periods (12–48 hr for con A-inducible IL-4 and 72–120 hr for the spontaneous production of IFN-γ and IL-10 in the absence of con A). Results from a single representative experiment are displayed as mean cytokine concentration in ng/ml ± SD, where standard deviations are displayed only when they exceed 0·5 ng/ml (IL-4) or 0·2 ng/ml (IL-10 and IFN-γ). Values below limit of detection not shown.

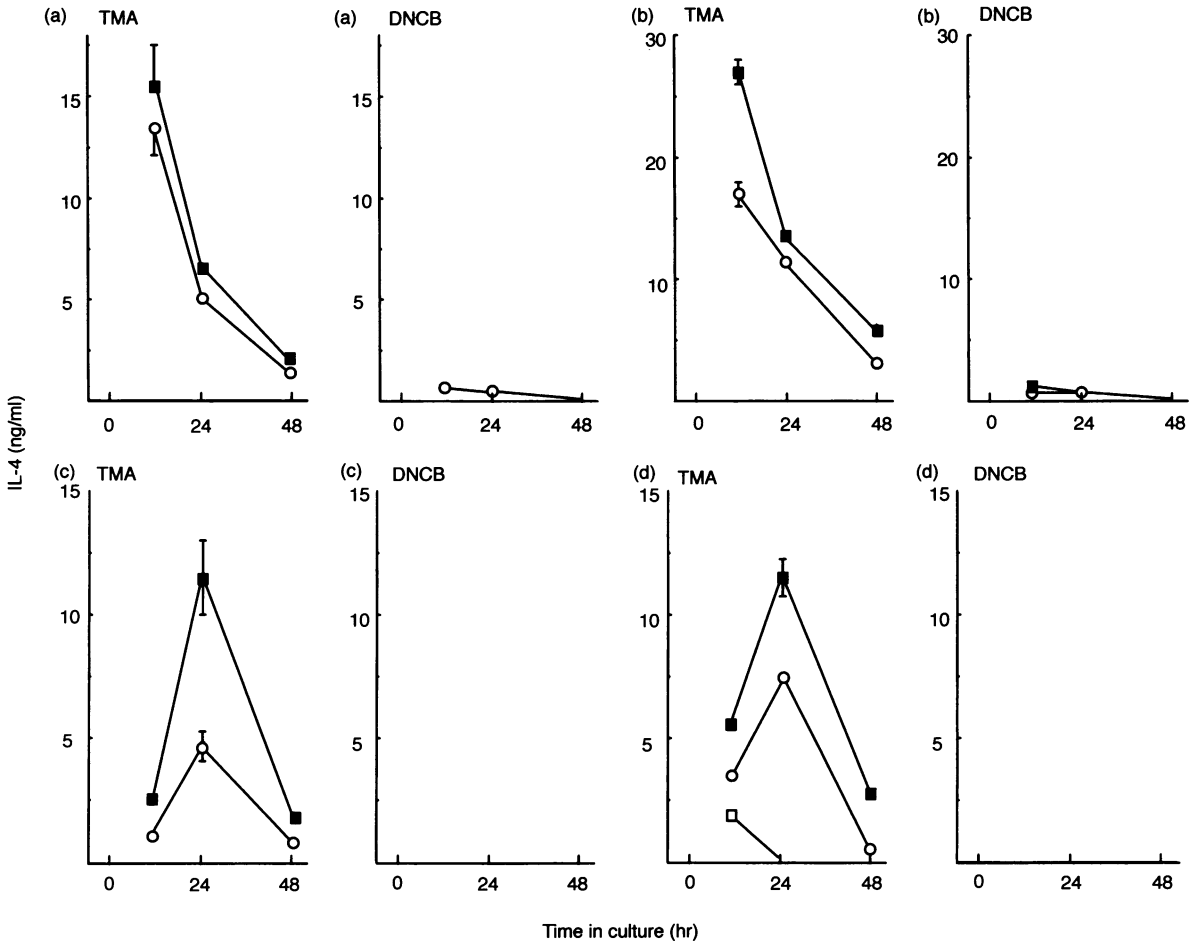

Figure 2. Contribution of CD4⁺ and CD8⁺ T lymphocytes to the mitogen-inducible production of IL-4 by draining LNC prepared from mice sensitized with TMA or DNCB. LNC were isolated from mice following chronic topical exposure to 10% TMA or 1% DNCB. Cells were treated with complement and an isotype control preparation of IgG2b rat myeloma protein (○), anti-CD4 antibody (□) or anti-CD8 antibody (■). IL-4 concentration was measured by ELISA in supernatants prepared following culture of LNC for 12–48 hr in the presence of 2 μg/ml con A. Results from four independent experiments A–D are shown as mean IL-4 concentration in ng/ml ± SD, where standard deviations are displayed only when they exceed 0·5 ng/ml IL-4. Values below limit of detection are not shown.

Contribution of CD4⁺ and CD8⁺ T lymphocytes to LNC cytokine production profiles

In all experiments, depletion of CD4⁺ or CD8⁺ T lymphocytes with the appropriate antibody and complement was very effective (>98% depletion) as judged by subsequent fluorescence analysis of the residual populations. The results of four independent experiments investigating cytokine production are summarized in Figs 2, 3 and 4. In each of these draining LNC prepared from TMA-treated mice produced high levels of mitogen-inducible IL-4 that was maximal at either 12 or 24 hr of culture. In all instances, depletion of CD4⁺ cells resulted in the complete, or almost complete, loss of IL-4 secretion (Fig. 2). In contrast, depletion of CD8⁺ cells caused either no change in the production of IL-4 or an increase (Fig. 2). Draining LNC prepared from DNCB-sensitized mice produced undetectable or only very low concentrations of IL-4 and this prohibited evaluation of the relative contribution of CD4⁺ and CD8⁺ cells (Fig. 2).

LNC from TMA-sensitized mice also produced high levels of IL-10, in this case with maximal activity following either 96 or 120 hr of culture. In common with IL-4, treatment of LNC with anti-CD4 and complement resulted in either a very significant reduction in, or complete loss of, IL-10 secretion (Fig. 3). Much lower levels of IL-10 were found in culture supernatants prepared from LNC isolated from DNCB-sensitized animals. In three of four experiments it was apparent, however, that production of IL-10 by LNC from DNCB-treated mice was dependent upon the presence of CD4⁺ T lymphocytes (Fig. 3).

A rather different picture was observed when supernatants were analysed for IFN-γ. Consistent with the data displayed in Fig. 1, treatment of mice with DNCB resulted in the production by draining LNC of comparatively high levels of IFN-γ, with maximal activity evident 120 hr following culture. In three of four experiments it was found that depletion of either CD4⁺ or CD8⁺ cells resulted in a reduction in IFN-γ secretion; in two instances the effect of CD4⁺ cell depletion being greater and in one instance depletion of CD8⁺ cells having the greater impact. In the other experiment only depletion of CD4⁺ cells was found to have any influence on IFN-γ production, and then only at a single time point (Fig. 4). Lower levels of IFN-γ secretion were observed following culture of LNC from TMA-sensitized mice. In each of the experiments illustrated in Fig. 4 it was found that depletion of

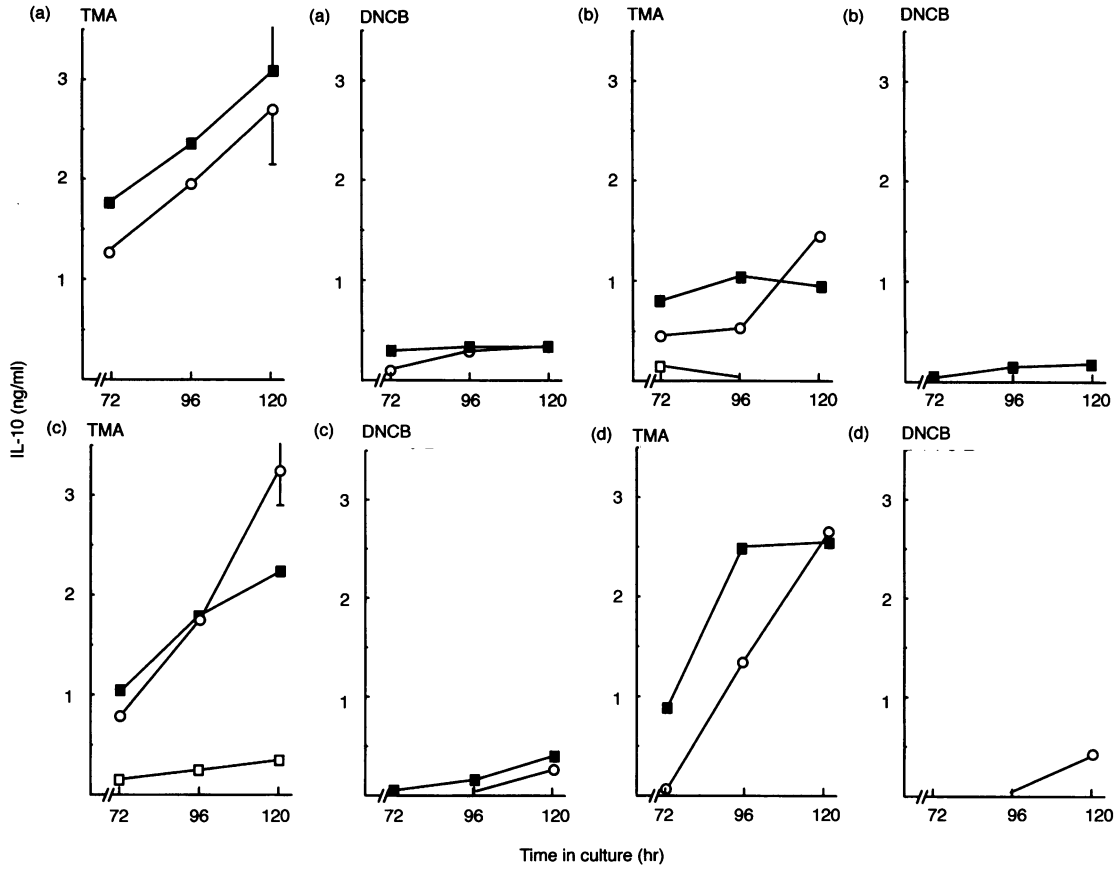

Figure 3. Contribution of $CD4^+$ and $CD8^+$ T lymphocyte to the production of IL-10 by draining LNC prepared from mice sensitized with TMA or DNCB. LNC were isolated from mice following chronic topical exposure to 10% TMA or 1% DNCB. Cells were treated with complement and an isotype control preparation of IgG2b rat myeloma protein (○), anti-CD4 antibody (□) or anti-CD8 antibody (■). IL-10 concentration was measured by ELISA in supernatants prepared following culture of LNC for 72–120 hr. Results from four independent experiments A–D are shown as mean IL-10 concentration in ng/ml ± SD, where standard deviations are displayed only when they exceed 0·2 ng/ml IL-10. Values below limit of detection are not shown.

$CD8^+$ cells caused a very substantial reduction in the comparatively low levels of IFN-γ produced by LNC from TMA-treated animals. Depletion of $CD4^+$ cells resulted in increased IFN-γ secretion compared with control populations treated with complement alone (Fig. 4).

Contribution of $CD4^+$ and $CD8^+$ T lymphocytes to cytokine production profiles of LNC from mice sensitized with MDI or formaldehyde

Experiments were conducted with a second pair of chemical allergens to determine whether the changes in cytokine secretion patterns observed following depletion of $CD4^+$ or $CD8^+$ cells were restricted to sensitization with TMA or DNCB. The production of IL-10, IFN-γ and mitogen-inducible IL-4 were measured following culture of draining LNC prepared from mice sensitized with either MDI or formaldehyde. The results of a single representative experiment are illustrated in Fig. 5. In common with TMA, the chemical respiratory allergen MDI induced, with similar kinetics, the production by LNC of relatively high concentrations of IL-10 and con A-inducible IL-4. Depletion of $CD8^+$ cells was without effect on the production of these cytokines. Conversely, removal of $CD4^+$ lymphocytes resulted in the complete loss of both IL-4 and IL-10 secretion (Fig. 5). Exposure of mice to formaldehyde failed to stimulate the production by draining LNC of detectable mitogen-inducible IL-4. Some production of IL-10 was observed and here again depletion of $CD4^+$ cells resulted in a complete loss of activity, whereas removal of $CD8^+$ cells was without influence (Fig. 5). Like DNCB, formaldehyde provoked the secretion by draining LNC of comparatively high concentrations of IFN-γ. Production of this cytokine was reduced substantially, but not lost completely, following depletion of $CD4^+$ cells. Only low levels of IFN-γ secretion were observed with LNC from mice treated with MDI and depletion of $CD8^+$ cells caused loss of activity (Fig. 5).

DISCUSSION

The data reported here suggest a complicated picture of the relative contributions of $CD4^+$ and $CD8^+$ T lymphocytes to the cytokine secretion profiles induced in mice by repeated exposure to respiratory or contact chemical allergens. Certain conclusions can, however, be drawn.

It is apparent that the production of IL-10 and of mitogen-inducible IL-4 by draining LNC stimulated by exposure of mice to chemical respiratory allergens such as TMA and MDI is dependent upon the presence of $CD4^+$ cells. In each instance depletion of

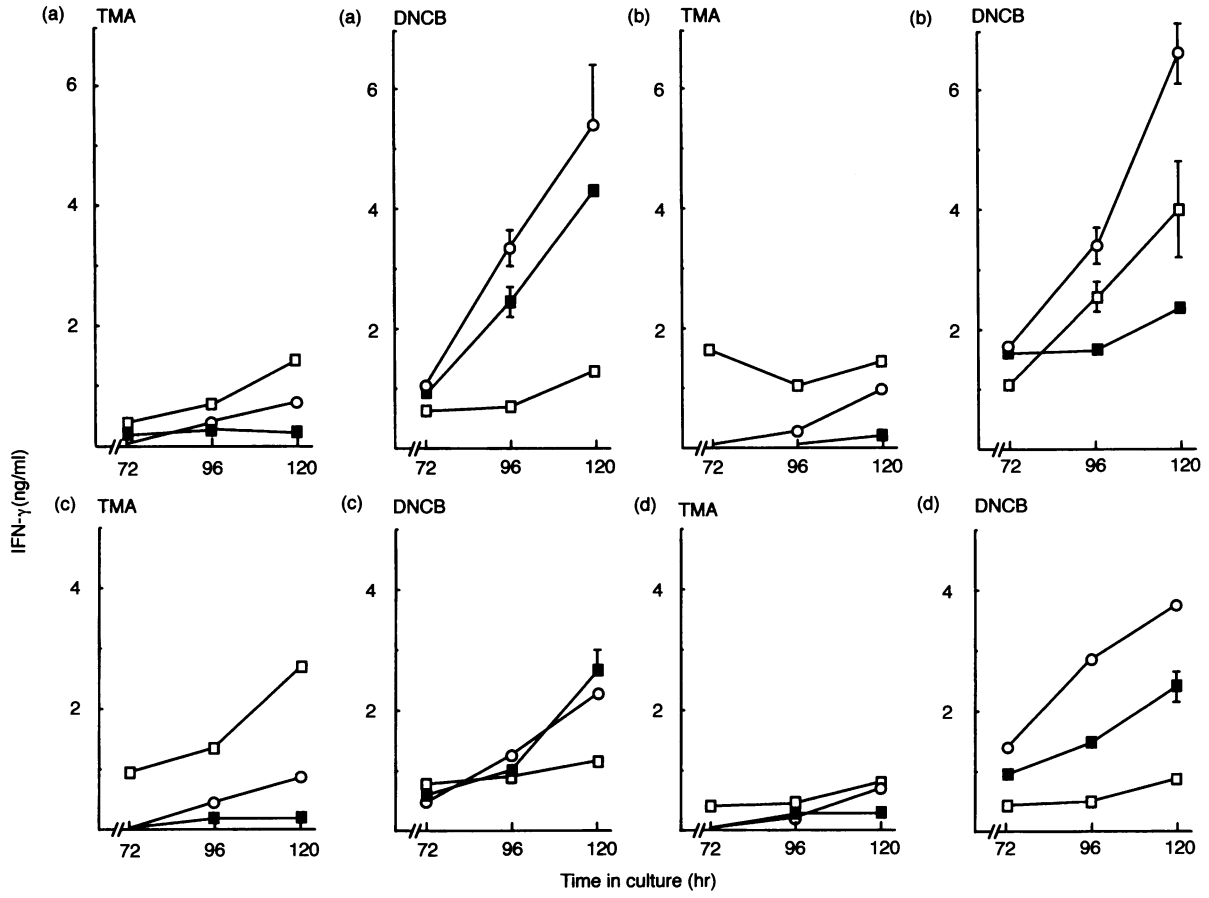

Figure 4. Contribution of CD4⁺ and CD8⁺ T lymphocytes to the production of IFN-γ by draining LNC prepared from mice sensitized with TMA or DNCB. LNC were isolated from mice following chronic topical exposure to 10% TMA or 1% DNCB. Cells were treated with complement and an isotype control preparation of IgG2b rat myeloma protein (○), anti-CD4 antibody (□) or anti-CD8 antibody (■). IFN-γ concentration was measured by ELISA in supernatants prepared following culture of LNC for 72–120 hr. Results from four independent experiments A–D are shown as mean IFN-γ concentration in ng/ml ± SD, where standard deviations are displayed only when they exceed 0·2 ng/ml IFN-γ. Values below limit of detection are not shown.

CD4⁺ cells resulted in a very substantial reduction in, or a complete loss of, both IL-4 and IL-10 secretion. The production of these cytokines was either unchanged or enhanced in cultures depleted of CD8⁺ cells; such differences being attributable presumably to changes in the relative number and/or relative activity of CD4⁺ and CD8⁺ T lymphocytes in individual preparations. The contact allergens DNCB and formaldehyde induced the production of only very low levels of IL-4 and IL-10 and in these instances the relative contributions of CD4⁺ and CD8⁺ cells were difficult to assess. However, in those cases where production of type 2 cytokines was sufficient for an evaluation to be made then it appeared that depletion of CD4⁺ cells resulted in a complete loss of IL-4 and IL-10 secretion. In contrast to IL-4 and IL-10, the production by draining lymph node cells of IFN-γ appeared to be less dependent upon the presence of CD4⁺ cells. DNCB and formaldehyde both induced relatively vigorous secretion of IFN-γ. The results indicate that both CD4⁺ and CD8⁺ cells contribute to the secretion of this cytokine in contact sensitized mice with the former usually, but not invariably, making the greater contribution. The respiratory allergens TMA and MDI induced much lower levels of IFN-γ production. The data described here suggest that the major or sole source of the low concentrations of IFN-γ produced by LNC prepared from mice exposed to respiratory chemical allergens is CD8⁺ cells.

Taken together, the interpretation is that CD4⁺ T lymphocytes are the source of the type 2 cytokine (IL-4 and IL-10) responses stimulated in mice by exposure to either contact or respiratory chemical allergens. It can not be formally excluded, however, that CD8⁺ T lymphocytes may also represent a source of IL-4 and IL-10, but that their production of these cytokines is dependent upon the continued presence of CD4⁺ cells. Resolution of this issue will require the analysis of LNC prepared by positive, rather than negative, selection techniques and the conduct of repletion experiments and the use of *in situ* techniques. Irrespective of this, it is clear that a different situation exists with respect to the production of IFN-γ. Both CD4⁺ and, to a lesser extent, CD8⁺ T cells appear to contribute to the production of this cytokine by LNC prepared from mice exposed to DNCB, while CD4⁺ T cells represent the exclusive source of IFN-γ from formaldehyde-activated LNC. In the case of respiratory allergens the data available suggest that CD8⁺ cells are the major source of the low levels of IFN-γ produced.

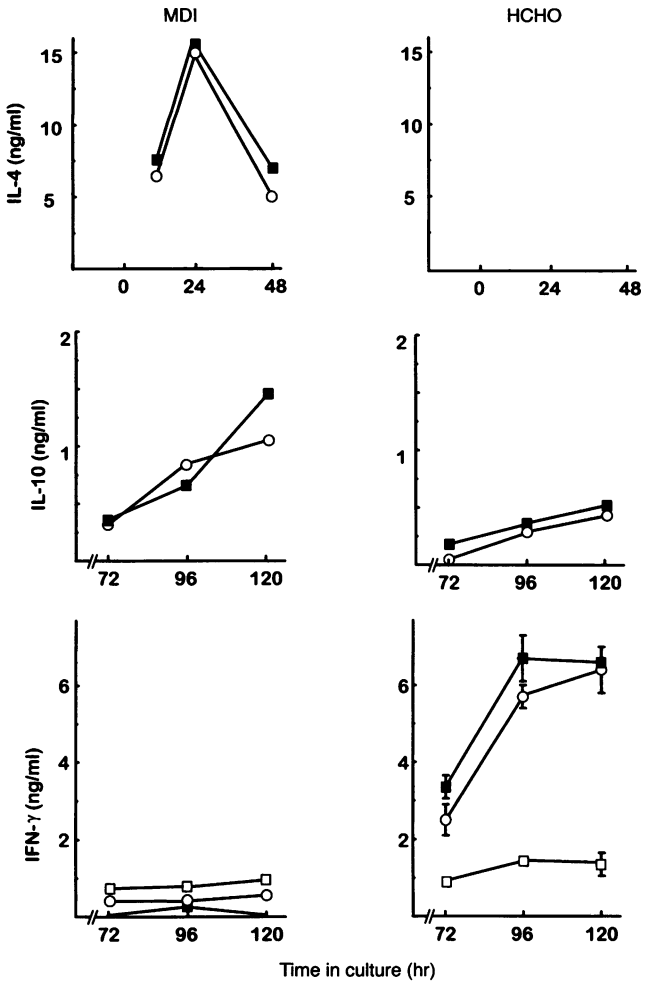

Figure 5. Contribution of CD4$^+$ and CD8$^+$ T lymphocytes to the cytokine production profiles of draining LNC prepared from mice sensitized with MDI or formaldehyde. LNC were isolated from mice following chronic topical exposure to 1% MDI or 50% formaldehyde (HCHO). Cells were treated with complement and an isotype control preparation of IgG2b rat myeloma protein (○), anti-CD4 antibody (□) or anti-CD8 antibody (■). Cytokine concentrations were measured by ELISA following culture of LNC in the presence of 2 μg/ml con A for 12–48 hr (for IL-4 production) or in the absence of con A for 72–120 hr (for the production of IL-10 and IFN-γ). Results from a single representative experiment are shown as mean cytokine concentration in ng/ml ± SD, where standard deviations are displayed only when they exceed 0·5 ng/ml (IL-4) or 0·2 ng/ml (IL-10 and IFN-γ).

The inference is that CD8$^+$ T lymphocytes represent a source, and sometimes possibly the most important or sole source, of IFN-γ in the draining lymph nodes of mice sensitized to chemical respiratory allergens. If such is the case then the selective stimulation of Th2-type cells in mice by respiratory sensitizers may be more absolute than suspected previously, with the induction of only Th2-like and Tc1-type responses. The corollary is that contact allergens such as DNCB and formaldehyde stimulate the development of Th1 and, to a lesser extent, Th2 cells together with Tc1-type responses. Notwithstanding the relative contribution made to the cytokine environment by CD4$^+$ and CD8$^+$ T lymphocytes, the overall characteristics of the responses induced in mice by contact and respiratory chemical sensitizers reflect their allergenic activity. Thus, respiratory allergens stimulate IgE responses, whereas contact allergens that are considered not to induce respiratory sensitization fail to provoke IgE antibody production.[11,12]

It is known that CD8$^+$ Tc1-type cells inhibit allergen-induced airways hyperresponsiveness in mice and rats.[22,23] There is also growing evidence that these same cells play a role of considerable importance in the negative regulation of IgE antibody responses.[22,23,29–31] It is assumed that the production of IFN-γ by such Tc1-type cells is one important mechanism through which CD8$^+$ lymphocytes are able to regulate negatively IgE responses. This may not be the sole mechanism, however. It has been reported recently that the capacity of CD8$^+$ cells to inhibit IgE responses to inhaled aeroallergens is largely or wholly attributable to a minority population of extremely active CD8$^+$ lymphocytes which display γδ T-cell receptors.[32,33] Transfer to syngeneic recipients of as few as one thousand CD8$^+$ γδ$^+$ T lymphocytes from rats repeatedly exposed to antigen by inhalation was found to cause antigen-specific and IgE isotype-specific tolerance.[33] Whether similar regulatory CD8$^+$ T cells are found among allergen-activated lymph nodes of mice exposed topically to chemical sensitizers is presently unknown.

CD8$^+$ cells of Tc1 phenotype may play roles in chemical sensitization other than the active regulation of IgE responses. There is mounting evidence that CD8$^+$ T lymphocytes are induced by contact allergens and may contribute significantly to contact hypersensitivity responses, the relevant effector population presumably being IFN-γ-producing Tc1-type cells.[24,34–36] Indeed, it is proposed in mice that the effectiveness of contact hypersensitivity reactions may represent the balance between effector CD8$^+$ cells and negative regulatory Th2-type CD4$^+$ cells.[24,36] It is likely, therefore, that the stimulation of Tc1-type CD8$^+$ responses, as described here, will favour the induction and expression of contact sensitization, but if such cells are present in sufficient numbers they will serve to inhibit IgE responses and immediate-type allergic reactions.

It is not yet clear what factors drive the maturation of CD8$^+$ precursors toward phenotypes of selective cytokine secretion. What evidence there is suggests, however, that in common with Th cells, differentiated Tc populations develop in response to cytokine signals. Thus, interleukin 12 (IL-12) and IFN-γ encourage the differentiation of precursors into Th1 or Tc1 cells, whereas IL-4 favours the development of Th2 or Tc2 cells.[18,20,21,37] The source of the cytokines that provide the initial stimulus for the development of selective Th and/or Tc responses remains controversial. While IFN-γ and IL-12 derived, respectively, from natural killer (NK) cells, and dendritic cells and macrophages presumably provide the important signals for Th1/Tc1 development,[38] a number of potential sources of IL-4 has been proposed. These include mast cells, NK1.1,$^+$ CD4$^+$ T cells and γδ T lymphocytes.[39–42]

Whatever the nature of the cells involved in early cytokine production and irrespective of the costimulatory signals that favour the selectivity of T lymphocyte responses, it assumed that chemical respiratory allergens such as TMA and MDI provoke the stimuli necessary to drive the differentiation of T helper cells toward a preferential, or sometimes possibly exclusive, Th2-type phenotype. From the data presented here it is suggested that what little type 1 responsiveness such chemicals induce is embodied in Tc1-type CD8$^+$ T lymphocytes, but that the inhibitory properties of

these cells are outweighed by the promotional signals of Th2 products, such that IgE responses are generated and maintained and immediate-type allergic responses favoured. In contrast, it is suggested that strong contact allergens induce both Th1- and Tc1-type responses and that these cells, acting either alone or in concert, are responsible for effecting contact hypersensitivity reactions. In such circumstances the production of IFN-γ by Th1 and Tc1 cells outweighs the small amounts of IL-4 produced as the result of a less vigorous Th2-type response, thereby creating conditions that are non-permissive for IgE antibody production.

In summary, the quality of immune response induced by chemical allergens is likely to be determined by the relative contribution of functional subpopulations of both $CD4^+$ and $CD8^+$ T lymphocytes.

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