Interferon-γ Inducible Protein (IP-10) Expression Is Mediated by CD8$^+$ T Cells and Is Regulated by CD4$^+$ T Cells During the Elicitation of Contact Hypersensitivity

Mihoko Abe,*[1] Tsunenori Kondo,†[1] Hui Xu,*[1] and Robert L. Fairchild*†

Departments of *Immunology and †Urology, Cleveland Clinic Foundation, Cleveland, Ohio, U.S.A.

To investigate the potential roles of CD4$^+$ and CD8$^+$ T cells during contact hypersensitivity, we examined the T-cell–dependent expression of proinflammatory cytokine genes in the responses to dinitrofluorobenzene and oxazolone. Whole cell RNA was isolated from challenged ear tissue and analyzed for level of cytokine gene expression by Northern blot and densitometry analysis. Expression of interleukin 1β and the three chemokine genes (IP-10, JE, and KC) examined was dependent on the hapten dose used for sensitization and correlated with the immune response, i.e., ear swelling, elicited. Antibody-mediated depletion of CD8$^+$ T cells before sensitization resulted in the absence of IP-10 expression following hapten challenge, indicating the ability of immune CD8$^+$ T cells to mediate IP-10 expression. Depletion of CD4$^+$ T cells resulted in higher levels of IP-10 and KC expression during elicitation of contact sensitivity, suggesting CD4$^+$ T cells inhibit the expression of these proinflammatory genes. Depletion of CD4$^+$ T cells resulted in contact hypersensitivity responses of higher magnitude and depletion of CD8$^+$ T cells resulted in responses of lower magnitude. Transfer of CD8$^+$ T-cell–depleted immune cells resulted in low, but detectable levels of IP-10 expression, indicating the ability of some oxazolone-immune CD4$^+$ T cells to mediate IP-10 expression. These results indicate the differential induction of proinflammatory cytokine gene expression during elicitation of contact hypersensitivity in which expression of IP-10 is primarily mediated by immune CD8$^+$ T cells and inhibited by immune CD4$^+$ T cells. ***Key words: chemokines/skin inflammation/CD-8 T-cell response. J Invest Dermatol 107:360–366, 1996***

Chemokines are low molecular weight proteins that play a major role in leukocyte trafficking to tissue sites of inflammation (reviewed in Oppenheim *et al*, 1991; Miller and Krangel, 1992). The chemokines have been divided into two families based on the position of the first two of four conserved cysteine residues. The α (C-X-C) chemokine family includes interleukin-8 (IL-8), interferon-γ inducible protein (IP-10), and GROα, and its murine homolog, KC (Luster and Ravetch, 1987; Baggiolini *et al*, 1989; Watanabe *et al*, 1989). The β (C-C) chemokine family includes macrophage inflammatory protein-1α, macrophage inflammatory protein-1β, and monocyte chemotactic protein-1 and its murine homolog, JE (Sherry *et al*, 1988; Matsushima *et al*, 1989; Rollins *et al*, 1990). *In vitro*, various chemokines have been shown to stimulate increased integrin expression and cell adhesion, as well as chemotaxis of target cells (Schall *et al*, 1993; Taub *et al*, 1993a,b; Vaddi and Newton, 1994). Many different chemokines or their transcripts have been detected during analyses of various inflammatory reactions (Kaplan *et al*, 1987; Ransohoff *et al*, 1993; Devergne *et al*, 1994). A recent study using anti–macrophage inflammatory protein-1α antibody to ameliorate cellular infiltration and pathology in the bleomycin induced lung injury model is consistent with the important role of chemokines in inflammatory disease (Smith *et al*, 1994). Although these studies indicate the production of chemokines during inflammation, the role of T cells in inducing and regulating chemokine production during immune responses remains poorly defined.

Although the expression of chemokine genes during the induction or sensitization phase of contact hypersensitivity (CHS) has been well characterized (Barker *et al*, 1990, 1991; Enk and Katz, 1992; Gautam *et al*, 1994), the expression of these genes during the T-cell–mediated elicitation phase of the response remains unexplored. Because T-cell–derived cytokines induce the expression of chemokine genes (Hamilton *et al*, 1989; Barker *et al*, 1990; Narumi *et al*, 1992), we hypothesized that different subsets of hapten/major histocompatibility complex (MHC)–specific T cells participating in the CHS response would induce different patterns of chemokine gene expression. We have tested this hypothesis by examining the expression of three chemokine genes (IP-10, JE, and KC) following hapten challenge of sensitized animals depleted of CD4$^+$ and/or CD8$^+$ T cells by *in vivo* administration of specific monoclonal antibody. The results support our hypothesis in that the magnitude of IP-10 gene expression observed during the CHS responses to 2,4-dinitrofluorobenzene (DNFB) and oxazolone (Ox) is depen-

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Reprint requests to: Dr. Robert L. Fairchild, Department of Immunology, FFb-41, Research Institute, Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44195-0001.

[1]The first three authors contributed equally to this work.

Abbreviations: CHS, contact hypersensitivity; LNC, lymph node cells; Ox, oxazolone.

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dent upon the activities of primed $CD4^+$ and $CD8^+$ T cells. The expression of IP-10 during CHS is primarily mediated by immune $CD8^+$ T cells, and immune $CD4^+$ T cells inhibit the level of IP-10 expression. These results indicate an important difference between CHS and classical delayed type hypersensitivity (DTH) reactions where $CD4^+$ T cells induce, rather than inhibit, IP-10 expression.

MATERIALS AND METHODS

Mice BALB/c mice were obtained through Dr. Clarence Reeder at the National Cancer Institute (Frederick, MD). Adult females of 6–10 wk of age were used throughout this study.

Antibodies mAb from the culture supernatant of the rat IgG-producing hybridomas YTS 191.1.2 and YTS 169.4.2.1 (anti-mouse CD4 and CD8 antibodies, respectively) (Cobbold *et al*, 1984) were purified by protein G chromatography.

Sensitization and Elicitation of Contact Hypersensitivity Mice were sensitized and challenged to elicit CHS responses to DNFB and Ox as previously described by Moorhead (1977). For the induction of CHS to DNFB, groups of three mice were sensitized by two daily paintings (days 0 and +1) with 25 μl of 0.5% DNFB (Sigma Chemical, St. Louis, MO) on the shaved abdomen and 5 μl on the footpads. For the induction of CHS to Ox, groups of three or four mice were painted on the shaved abdomen once with 50 μl of 3% Ox (Aldrich Chemical, Milwaukee, WI) and 10 μl on the footpads. On day +5, the ear thickness of sensitized and unsensitized control animals was measured with an engineer's micrometer (Mitutoyo, Japan) and each animal was challenged by applying 10 μl of 0.2% DNFB or 1% Ox to each side of both ears. Increase in ear swelling (Δ) was measured in a blinded manner 24 h after challenge and expressed in units of 10^{-4} in. as previously described (Fairchild *et al*, 1993). The magnitude of ear swelling is given as the mean increase of each group of three individual animals (i.e., six ears) ± SEM. The statistical significance in ear swelling responses between experimental groups of mice was determined using Student's t test.

Antibody Treatment for Depletion of T Cells *In vivo*, $CD4^+$ and/or $CD8^+$ T cells were depleted by i.p. injection of 100 μg of YTS 191 (anti-CD4) and/or YTS 169 (anti-CD8) on 3 consecutive days as described by Cobbold and co-workers (1984). In our hands, this treatment resulted in depletion of ≥96% of the target T-cell population as assessed by flow cytometry analysis (data not shown). Antibody-treated mice were rested 1–3 d before shaving and hapten sensitization.

The *in vitro* strategy for depletion of $CD4^+$ or $CD8^+$ T cells by treatment with specific antibody and complement and transfer to naïve recipients was adapted from the procedure described by Gocinski and Tigelaar (1990). On day +4 after sensitization with Ox, lymph node cell suspensions were prepared and suspended at 50×10^6 per ml with 10 μg YTS 191 per ml (anti-CD4), YTS 169 (anti-CD8), or control (rat IgG) antibody in RPMI 1640. After 45 min on ice, the cells were washed, resuspended at 10^8 per ml in rabbit complement (Cedarlane, Hornby, Ontario, Canada), and incubated for 45 min at 37°C. Following extensive washing, the cells were counted and cell viability assessed. Aliquots of cells from each treated population were stained with anti-CD4, anti-CD8, or anti-rat IgG antibody and analyzed by flow cytometry. The cell viability and percentages of $CD4^+$ and $CD8^+$ T cells in each population were used to calculate the number of $CD4^+$ or $CD8^+$ T cells, and these numbers were used to transfer equivalent numbers of $CD4^+$ and/or $CD8^+$ T cells to four naïve mice per group in the control-treated and antibody-depleted groups.

Assessment of Gene Expression in Mouse Ear Tissue Gene expression in hapten-challenged ear tissue was examined by northern blot analysis of whole cell RNA, isolated from homogenized ear tissue. Briefly, both ears of all mice in each group were excised and homogenized in 4 M guanidine isothiocyanate using a Polytron homogenizer (Brinkmann, Westbury, NY). The tissue homogenate was layered over a 5.7 M $CsCl_2$ gradient and the RNA pelleted by centrifugation as described by Chirgwin and co-workers (1979). Following resuspension in diethyl pyrocarbonate-treated dH_2O, 15-μg aliquots of RNA were electrophoresed in 1% agarose formaldehyde-denaturing gels and analyzed by northern blot analysis as previously described (Fairchild *et al*, 1993). Northern blots were probed by hybridization with ^{32}P-labeled oligonucleotide probes specific for murine IL-1β (Ohmori *et al*, 1990), IP-10 (Ohmori and Hamilton, 1990), JE (Rollins *et al*, 1988), and KC (Introna *et al*, 1987). The quantity of RNA in each analysis was standardized by washing the blot 3× in Tris(hydroxymethyl)-aminomethane–ethylenediamine tetraacetic acid (Tris-EDTA) at 90°C to strip off the cytokine probe and reprobing the blot with an oligonucleotide probe specific for rat glyceraldehyde-3-phosphate dehydrogenase (GADPH; Fort *et al*, 1985). Densitometry was performed to measure the cytokine and GADPH signals for each sample of the blot, using blot exposures with autoradiographic signals in the linear range of the densitometer. The levels of chemokine gene expression elicited during CHS in different RNA samples were then normalized using the GADPH signal, set at a value of 1.0, and expressing the chemokine signal as a ratio of the GADPH signal. It should be noted that some blots were overexposed for photographic purposes and, therefore, the densitometric analyses give a more accurate comparison of the levels of proinflammatory cytokine gene expression between samples in an experiment. All experiments were repeated two or three times with similar results observed each time, and the results from a single representative experiment are shown.

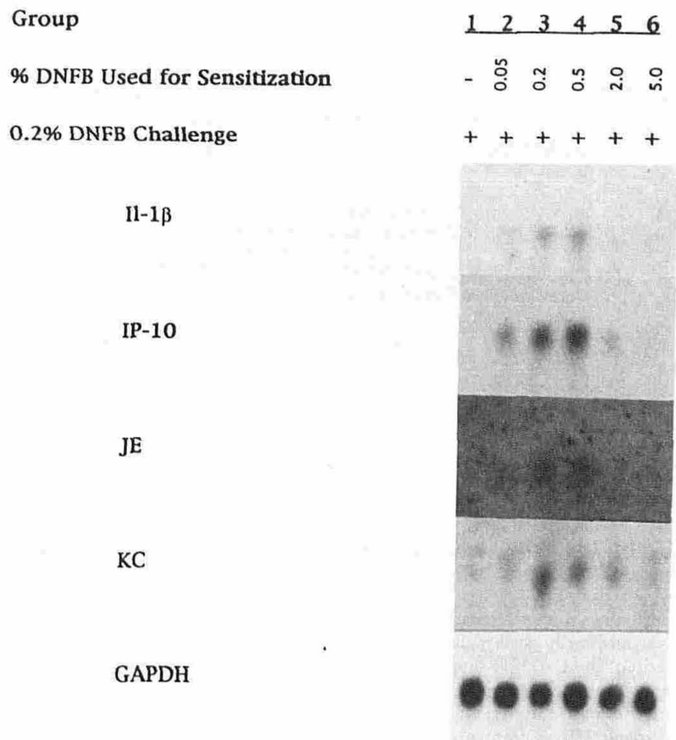

Figure 1. Expression of genes encoding proinflammatory cytokines during CHS to DNFB is sensitization dose dependent. BALB/c mice were sensitized by two daily (day 0 and +1) paintings with 25 μl of the indicated concentration of DNFB. At day +5, both ears of each animal were challenged with 0.2% DNFB, and 24 h later the ears were excised and total cellular RNA was prepared and analyzed by northern blot for expression of the indicated genes.

RESULTS

Concentration-Dependent Chemokine Gene Expression During the Elicitation of CHS to DNFB The influence of sensitizing hapten dose on the expression of chemokine genes 24 h after challenge with 0.2% DNFB was examined. Sensitization with 0.2–0.5% DNFB resulted in maximal levels of expression for IL-1β and the three chemokine (IP-10, JE, and KC) genes examined (**Fig 1**, Groups 3 and 4). Although the lower dose (0.05%) of DNFB resulted in decreased gene expression (Group 2), sensitization with supraoptimal doses (≥2%) nearly abrogated the expression of IP-10 and JE following hapten challenge (Groups 5 and 6). Low levels of KC, but not IL-1β, IP-10, or JE, expression were consistently observed in ear tissue of nonimmune mice challenged with DNFB (Group 1), indicating that sensitization was required for expression of IP-10 and JE. The T cells were not the source of this expression as concanavalin A stimulation of either DNFB or Ox immune T cells for 48 h did not induce chemokine expression (data not shown). Comparison of the level of chemokine gene expression by densitometry and the magnitude of immune response elicited by DNFB challenge indicated a strong correlation between the sensitization concentration-dependent expression of all four genes and

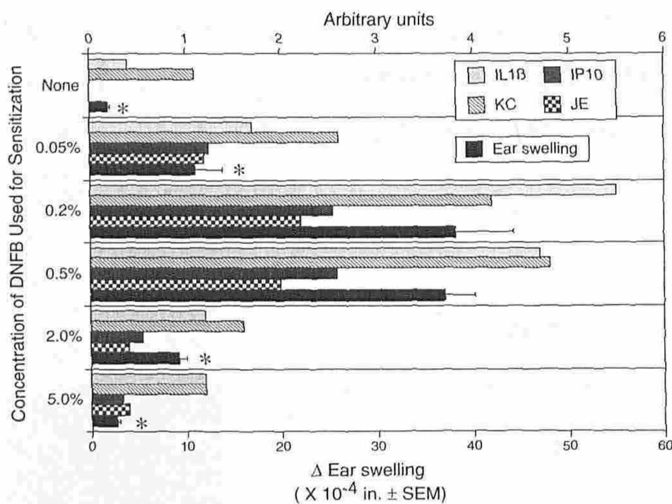

Figure 2. Chemokine gene expression correlates with ear swelling response during CHS to DNFB. Levels of chemokine gene expression observed in **Fig 1** were compared using the levels of GADPH expression to normalize the amount of RNA loaded into each well. The GADPH signal was arbitrarily set a value of 1.0 and the chemokine signal expressed as a ratio of the GADPH signal. Increase in ear swelling was measured 24 h after hapten challenge (which was immediately followed by excision of the ear for isolation of the RNA from the tissue) as described in *Materials and Methods*. When compared with the group sensitized with 0.5% DNFB, $^*p < 0.001$.

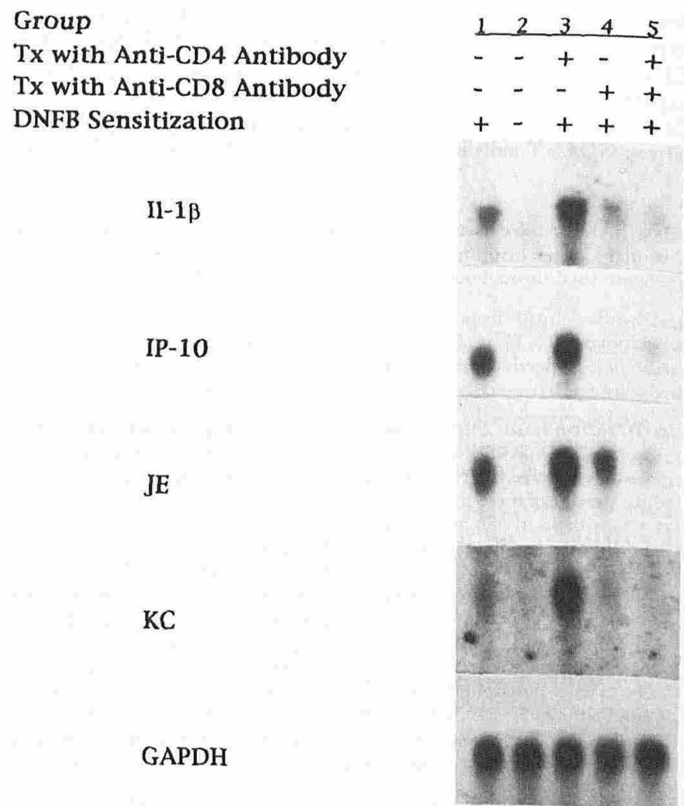

Figure 3. Expression of proinflammatory cytokine genes is increased during CHS to DNFB in the absence of CD4$^+$ T cells. Mice were depleted of CD4$^+$ and/or CD8$^+$ T cells by i.p. injection of 100 μg of monoclonal antibody on three consecutive days. Two days later the animals were sensitized with 0.5% DNFB. CHS was elicited by challenge of the ears with 0.2% DNFB, and 24 h later the ears were excised, total cellular RNA prepared and analyzed by northern blot for expression of the indicated genes.

the ear swelling response **(Fig 2)**. Temporally, maximal expression of all proinflammatory cytokine genes was observed at 24 h post-challenge except for KC at 12 h post-challenge (data not shown).

CD8$^+$ T-Cell–Mediated Expression of IP-10 During Elicitation of CHS to DNFB We next asked if the expression of chemokine genes during elicitation of the CHS response could be attributed to the activities of either immune CD4$^+$ or immune CD8$^+$ T cells. This was first tested using antibody-mediated depletion *in vivo*. Mice were treated with monoclonal anti-CD4 and/or anti-CD8 antibodies, resulting in almost complete depletion (≥96%) of the target T-cell population, and sensitized with 0.5% DNFB. Sensitized and control, unsensitized animals were hapten-challenged, and 24 h later the ear thickness was measured before the ears were removed for isolation of RNA and northern blot analysis. Expression of IL-1β and the three chemokine genes was detectable in the DNFB-challenged ear tissue of control antibody (rat IgG)-treated immune animals but not following challenge of unsensitized animals **(Fig 3**, Group 1 *vs* 2). Depletion of CD4$^+$ T cells resulted in elevated expression of IL-1β, IP-10, and KC, as well as control levels of JE (Group 3). Depletion of CD8$^+$ T cells resulted in the absence of IP-10 and low IL-1β and KC expression but control levels of JE expression (Group 4). Depletion of both CD4$^+$ and CD8$^+$ T cells resulted in very low to undetectable levels of gene expression following hapten challenge (Group 5), indicating that immune T-cell activity was required for proinflammatory cytokine gene expression during elicitation of CHS to DNFB.

The expression of IL-1β and the three chemokine genes observed in the experiment were normalized using the level of GADPH expression and compared with the magnitude of the CHS (i.e., ear swelling) response **(Fig 4)**. Depletion of CD4$^+$ T cells (Group 3) resulted in higher ear swelling responses to DNFB, as well as in increased expression of the four proinflammatory cytokine genes. Depletion of CD8$^+$ T cells (Group 4) resulted in the absence of IP-10, low KC expression, control levels of JE expression, and ear swelling responses that were not significantly above nonsensitized mice challenged with DNFB (Group 2).

CD8$^+$ T-Cell–Mediated Expression of IP-10 During the Elicitation of CHS to Ox Proinflammatory cytokine gene expression levels were examined in animals depleted of CD4$^+$ and/or CD8$^+$ T cells before sensitization and challenge with a different hapten, Ox **(Fig 5*a*,*b*)**. Similar to the DNFB response, the expression of IL-1β and the chemokines was readily apparent in ear tissue 24 h after challenge of Ox-sensitized mice (Group 1). Challenge of either Ox-immune animals with DNFB (data not shown) or nonimmune animals with Ox (Group 2) induced detectable expression of KC but not the other proinflammatory cytokine genes. As observed in the DNFB response, depletion of CD4$^+$ T cells before Ox sensitization resulted in increased expression of IL-1β, IP-10, and KC and control levels of JE during elicitation of the response (Group 3). Depletion of CD8$^+$ T cells prior to Ox sensitization and challenge resulted in the complete absence of IP-10 expression (Group 4). The levels of IL-1β, KC, and JE expression in the absence of CD8$^+$ T cells were almost identical to those observed during the response in the control antibody treated, Ox-immune group (Group 4 *vs* 1).

The levels of proinflammatory cytokine gene expression were normalized using the level of GADPH expression and compared with the ear swelling responses following Ox challenge **(Fig 6)**. In addition to the higher expression of IL-1β, IP-10, and KC in the challenged ears of immune mice depleted of CD4$^+$ T cells, a higher ear swelling response was consistently observed when compared with the control antibody-treated, hapten-sensitized mice (the Δ ear swelling for Group 1 was 138.94 ± 5.11 *vs* 162.06 ± 5.67 (mean ± SEM) for Group 3). Despite the absence of IP-10 expression, the Δ ear swelling response in Ox-sensitized mice

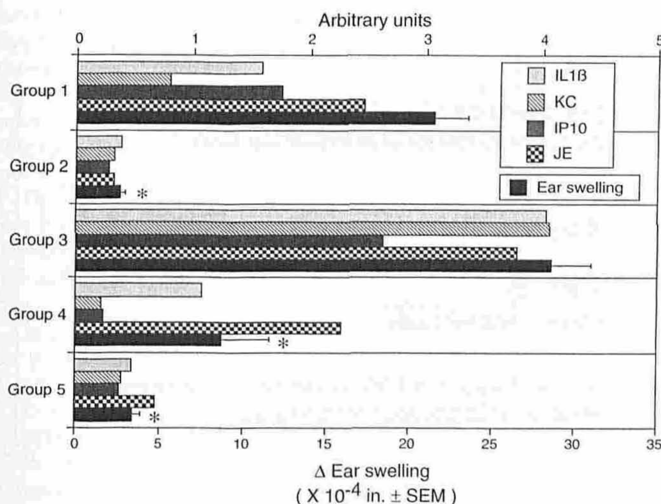

Figure 4. Chemokine gene expression correlates with the ear swelling response during elicitation of CHS to DNFB in mice depleted of CD4$^+$ and/or CD8$^+$ T cells. Levels of chemokine gene expression observed in **Fig 3** were compared using the levels of GADPH expression to normalize the amount of RNA loaded into each well. The GADPH signal was arbitrarily set a value of 1.0 and the chemokine signal expressed as a ratio of the GADPH signal. Increase in ear swelling was measured 24 h after DNFB challenge. When compared with control group 1, $^*p < 0.001$.

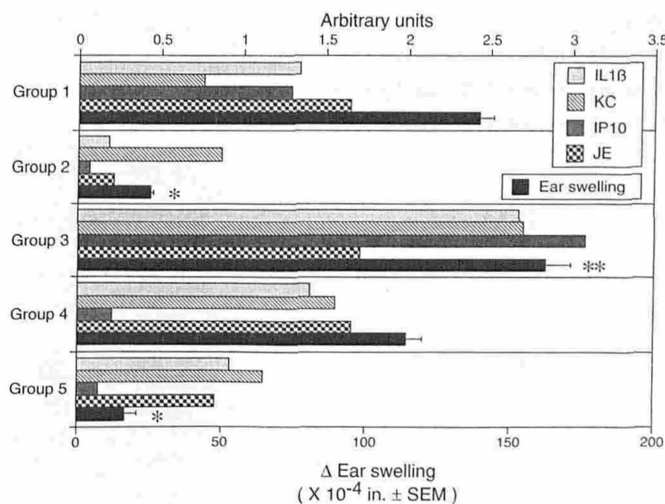

Figure 6. Chemokine gene expression correlates with the ear swelling response during elicitation of CHS to Ox in mice depleted of CD4$^+$ and/or CD8$^+$ T cells. Levels of chemokine gene expression observed in **Fig 5** were compared using the levels of GADPH expression to normalize the amount of RNA loaded into each well. The GADPH signal was arbitrarily set a value of 1.0 and the chemokine signal expressed as a ratio of the GADPH signal. Increase in ear swelling was measured 24 h after Ox challenge. When compared with control group 1, $^*p < 0.001$; $^{**}p < 0.05$.

depleted of CD8$^+$ T cells was 113.78 ± 5.67, a 20% reduction from the control (Group 1) response. It was consistently observed during these studies that the responses to Ox in control and CD8$^+$-depleted mice decreased rapidly after 24 h post-challenge and reached background levels by 4–5 d post-challenge, whereas the responses in CD4$^+$ T-cell–depleted animals decreased much more slowly and remained significantly above background levels until 10–12 d post-challenge (data not shown).

Chemokine Expression Mediated by Transferred Ox-Immune CD4$^+$ or CD8$^+$ T Cells To further examine the T-cell populations mediating proinflammatory cytokine gene expression during CHS to Ox, we transferred Ox immune lymph node cells (LNC) depleted of CD4$^+$ or CD8$^+$ T cells to naïve recipients and examined the levels of chemokine gene expression following hapten challenge. Immune LNC were depleted of CD4$^+$ or CD8$^+$ T cells by antibody and complement treatment and analyzed by flow cytometry as described in *Materials and Methods*. Flow cytometry analysis indicated ≤7% CD4$^+$ T cells in the anti-CD4 antibody plus complement-treated group and ≤2% CD8$^+$ T cells in the anti-CD8 antibody plus complement-treated group. Mice receiving control antibody and complement-treated LNC (**see Fig 8**, Group 1) received 50 × 10^6 total cells containing 20 × 10^6 CD4$^+$ T cells and 8 × 10^6 CD8$^+$ T cells per recipient; mice receiving CD8-depleted immune LNC (Group 3) received 40 × 10^6 total cells containing 20 × 10^6 CD4$^+$ T cells per recipient; and mice receiving CD4-depleted immune LNC (Group 4) received 40 × 10^6 total cells containing 8 × 10^6 CD8$^+$ T cells per recipient. One hour after cell transfer, the recipients, and nontransfer, control animals (Group 2), were challenged with Ox. After 24 h, the animals were sacrificed and the ear tissue was removed for whole cell RNA isolation and analysis by northern blot. As shown in **Figs 7** and **8**, challenge of recipients of control antibody and complement-treated immune LNC resulted in detectable expression of IL-1β and the chemokine genes whereas challenge of nontransfer control mice resulted in the induction of low expression of IL-1β and KC but not JE or IP-10 (Group 1 *vs* 2). In contrast to results observed following *in vivo* depletion of CD8$^+$ T cells, challenged ear tissue of recipients of CD8$^+$ T-cell–depleted immune LNC (Group 3) expressed low but detectable levels of IP-10, approximately one-third the level observed in the positive control response (Group 1). The expression levels of IL-1β and KC were slightly lower and the expression of JE was decreased by half during the response in recipients of

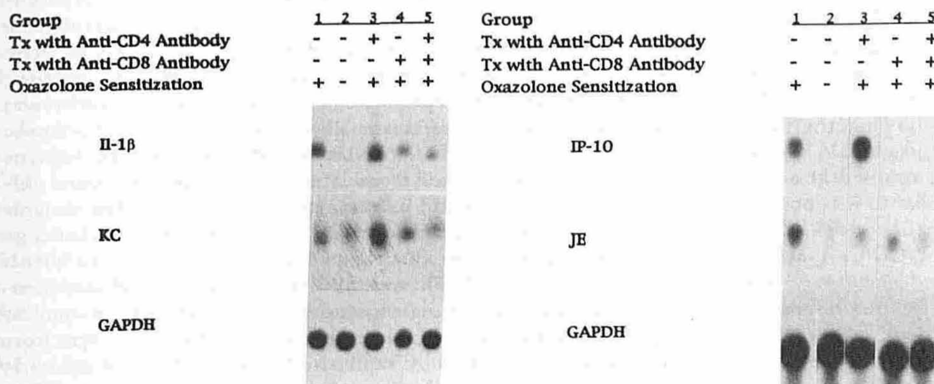

Figure 5. Expression of IP-10, KC, and IL-1β genes is amplified during CHS to Ox in the absence of CD4$^+$ T cells. Mice were depleted of T cells as in **Fig 4**. Two days later the animals were sensitized with 3% Ox. CHS was elicited by challenge of the ears with 1% Ox and 24 h later the ears were excised, total cellular RNA prepared and analyzed by northern blot for expression of the indicated genes.

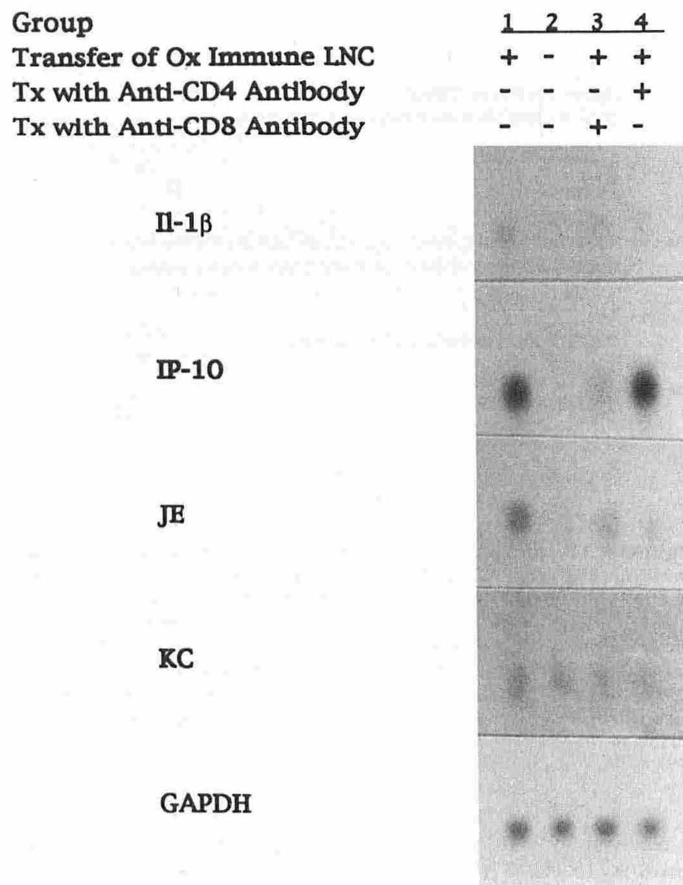

Figure 7. Ox-immune CD4$^+$ T-cell–mediated IP-10 expression is exposed during elicitation of passively transferred CHS. Four days after sensitization with 3% Ox, immune LNC suspensions were treated with control, rat IgG, (Group 1), anti-CD8 (Group 3), or anti-CD4 (Group 4) antibody and complement. Recipient groups (four mice/group) received equivalent numbers of CD4$^+$ and/or CD8$^+$ T cells, as described in the *Results*. All recipient and nontransfer control (Group 2) mice were ear challenged with 1% Ox, and 24 h later the ears were excised, and total cellular RNA prepared and analyzed by northern blot for expression of the indicated genes.

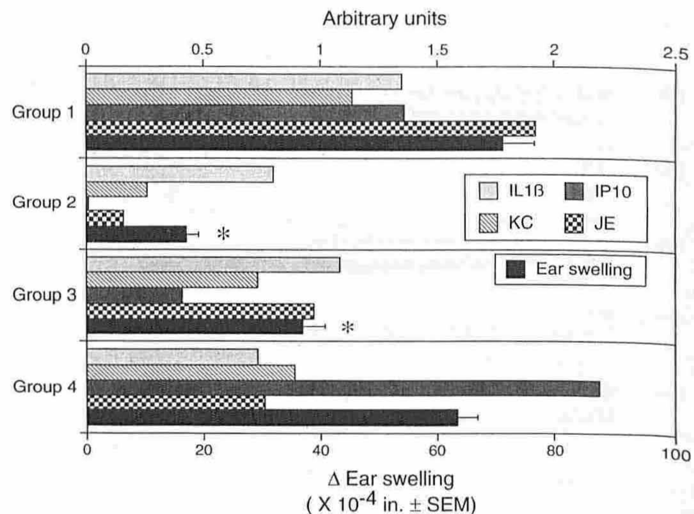

Figure 8. Chemokine gene expression correlates with ear swelling response during elicitation of passively transferred CHS to Ox. Levels of IL-1β and chemokine gene expression observed in **Fig 7** were compared using the levels of GADPH expression to normalize the amount of RNA loaded into each well. The GADPH signal was arbitrarily set a value of 1.0 and the chemokine signal expressed as a ratio of the GADPH signal. Increase in ear swelling was measured 24 h after Ox challenge. When compared with control group 1, *$p < 0.001$.

CD8$^+$ T-cell–depleted immune LNC when compared with the response in recipients of control-treated immune LNC. The transfer of CD8$^+$ T-cell–depleted immune LNC and recipient challenge resulted in an ear swelling response (36.83 ± 3.92, mean ± SEM) that was significantly lower than the response in recipients of control-treated immune LNC (Group 1, 71.29 ± 5.28) but significantly higher than the response in the nontransfer control (Group 2, 17.21 ± 2.11) 24 h after challenge. Consistent with the results observed in mice depleted of CD4$^+$ T cells by *in vivo* antibody treatment, hapten-challenged ear tissue from recipients of Ox-immune LNC depleted of CD4$^+$ T cells (Group 4) had increased levels of IP-10 expression, almost twice that observed in the positive control response. The levels of IL-1β, KC, and JE in these recipients were similar to those observed following transfer of CD8$^+$ T-cell–depleted immune cells. Depletion of CD4$^+$ T cells from the transferred immune cell population resulted in an ear-swelling response (63.39 ± 3.42) that was similar to that observed in recipients of control antibody and complement-treated immune cells. Although the responses in recipients of CD8$^+$ T-cell–depleted and control-treated immune cells reached background levels by 48 and 72 h post-challenge, respectively, the response in recipients of the CD4$^+$ T-cell–depleted immune cells remained significantly above background levels until 96 h post-challenge (data not shown).

DISCUSSION

Contact hypersensitivity has been used by many laboratories as a model to study the effector functions of T cells primed to hapten/MHC complexes. *In vitro* and *in vivo* antibody depletion studies have indicated the need for immune CD4$^+$ T cells for elicitation of CHS (Miller and Jenkins, 1985; Gautam *et al*, 1991). The generation of specific CD4$^+$ T-cell clones from the lesions of patients with allergic contact dermatitis to nickel and cobalt has supported the role of CD4$^+$ T cells in CHS (Sinigaglia *et al*, 1985; Lofstrom and Wigzell, 1986). These results have suggested that CHS is similar to classical DTH reactions in that CD4$^+$ T cells are the primary effector cells. Recently, Gocinski and Tigelaar (1990) used antibody-mediated *in vivo* depletion of CD4$^+$ and CD8$^+$ T cells to study effector mechanisms in CHS and observed the ability of CD8$^+$ T cells to mediate ear swelling responses to DNFB and Ox. Furthermore, the magnitude of the ear swelling response was negatively regulated by immune CD4$^+$ T cells although a CD4$^+$ T-cell effector component of the CHS response was also observed. Consistent with the effector role of CD8$^+$ T cells in CHS, the production of interferon-γ (IFN-γ) by urushiol-reactive CD8$^+$ T cells isolated from the skin lesions of patients sensitized to poison ivy has been reported (Kalish and Johnson, 1990).

In this report, we used a molecular approach to examine CD4$^+$ and CD8$^+$ T-cell function in CHS. Optimal expression of proinflammatory cytokine genes during elicitation of CHS was dependent on the activities of primed T cells and the levels of gene expression correlated with the magnitude of the ear swelling response observed. Except for KC, chemokine gene expression was not observed during hapten challenge of nonsensitized animals. The expression of IL-1β, IP-10, and JE observed in hapten-challenged trunk skin of recipients of trinitrochlorobenzene immune lymphoid cells and naïve animals was of equivalent magnitude 4 h after challenge, whereas expression at 24 h post-challenge was only observed in the skin of the immune cell recipients (Gautam *et al*, 1994). These results may indicate T-cell–independent expression of these genes at early times after hapten application, but it is also possible that the irritation of shaving the hair from the trunk skin followed by hapten application influenced this early expression. Recent studies using polymerase chain reaction (PCR)

have also indicated the expression of IL-1β, TNFα, and IL-10 shortly following hapten sensitization (Enk and Katz, 1992). Although these results indicated low-level gene expression during induction of CHS, our study has focused on elicitation of the response and indicates the high expression of proinflammatory cytokine genes mediated by T cells following challenge of immune animals.

The current data extend these studies by indicating that the pattern of chemokine gene expression during elicitation of CHS is dependent upon different T-cell subsets. Expression of the IFN-γ–induced chemokine IP-10 is absent in the hapten-challenged ear tissue of either naïve mice or hapten-sensitized mice depleted of $CD8^+$ T cells before sensitization, indicating that $CD8^+$ T cells are the primary mediators of IP-10 expression during CHS. The $CD8^+$ T-cell–dependent expression of IP-10 6 h after challenge during CHS to trinitrochlorobenzene has also been observed by Yuan and co-workers (1996). In contrast to the activity of $CD8^+$ T cells in CHS, immune $CD4^+$ T cells inhibit IP-10 expression. This was demonstrated by increased expression of IP-10 during the elicitation of CHS both in mice depleted of $CD4^+$ T cells by antibody treatment and in recipients following transfer of $CD4^+$-depleted immune cells. Because IFN-γ and tumor necrosis factor-α are cytokine mediators of CHS (Piguet *et al*, 1991; Enk and Katz, 1992) and inducers of IP-10 gene expression (Luster and Ravetch, 1987; Hamilton *et al*, 1989; Ohmori *et al*, 1993), our results suggest that hapten-specific $CD8^+$ T cells, rather than $CD4^+$ T cells, produce these cytokines during CHS. Stimulation of T cells from DNFB- and Ox-sensitized mice are supportive of this in that $CD8^+$ T cells produce IFN-γ and $CD4^+$ T cells produce IL-4 and IL-10 but no or little IFN-γ (Xu *et al*, 1996). The inhibition of IP-10 expression and production of IL-4 by hapten-primed $CD4^+$ T cells during CHS is consistent with observations by Gautam and co-workers (1992) indicating inhibition of cytokine-mediated expression of IP-10 in macrophages by IL-4. Because JE expression is induced by IL-4 and by IFN-γ (Rollins and Pober, 1991; Brown *et al*, 1994), the induction of JE by hapten-immune $CD4^+$ and $CD8^+$ T cells is consistent with the cytokine-producing phenotypes we have recently reported.

Through passive transfer of Ox-immune T cells, a segment of the $CD4^+$ T-cell population capable of inducing low level IP-10 expression was exposed. Challenge of recipients of Ox-immune $CD4^+$ T cells also resulted in a low ear swelling response of short duration. These results are consistent with those reported by Gocinski and Tigelaar (1990) in exposing an effector component within the hapten-immune $CD4^+$ T-cell population that has a largely negative regulatory function in CHS. One possible mechanism is that these CHS effector $CD4^+$ T cells mediate CHS through the production of cytokines other than IFN-γ and induce a low level inflammatory response. A second possible mechanism is the induction of a minor population of effector cells, producing IFN-γ and other proinflammatory cytokines, within the hapten-immune $CD4^+$ T-cell compartment. Three observations are consistent with the latter possibility: (i) transferred $CD4^+$ T cells from Ox-immune mice mediate low levels of IP-10 expression; (ii) in addition to the production of IL-4 and IL-10, we have consistently detected the production of small amounts of IFN-γ following stimulation of $CD4^+$ T cells from Ox-sensitized mice (Xu *et al*, 1996); and (iii) we have recently observed that the apparent but reduced ear swelling response mediated by Ox-immune $CD4^+$ T cells is completely abrogated by anti-IFN-γ antibody given at sensitization, indicating that the development of the CHS effector $CD4^+$ T cells is IFN-γ–dependent (DiIulio NA, Xu H, and Fairchild RL, unpublished observations). By contrast to the Ox response, we have not observed the ability of DNFB-immune $CD4^+$ T cells to mediate either IP-10 expression or passively transferred ear swelling responses (data not shown). An obvious difference in the Ox and DNFB responses is peak magnitude of the response in that the ear swelling response to Ox is usually 2- to 3-fold higher than the response to DNFB in our hands. Thus, it is possible that the strength of antigenicity may influence the phenotypic development of hapten-specific $CD4^+$ T cells to effector and regulatory cells during sensitization for CHS. Experiments to investigate this possibility are in progress.

Collectively, the results of this report, with those previously reported by Gocinski and Tigelaar (1990), reveal the development of $CD4^+$ and $CD8^+$ T-cell populations with different (and opposing) functional properties following sensitization with hapten contactants. The activation and production of cytokines by each of these cell populations may result in the generation of cutaneous sites with different inflammatory environments. The activities of immune $CD8^+$ T cells result in expression of the IFN-γ–induced chemokine IP-10 and optimal magnitude and duration of the CHS response. The activities of hapten-specific $CD4^+$ T cells primarily inhibit the expression of IP-10, as well as the magnitude and duration of the response. These results clearly distinguish CHS from DTH responses where T_H1 $CD4^+$ T cells produce IFN-γ following antigen challenge and induce, rather than inhibit, the expression of IP-10 (Mosmann *et al*, 1986; Kaplan *et al*, 1987).

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