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Expression and Participation of Eotaxin During Mycobacterial (Type 1) and Schistosomal (Type 2) Antigen-Elicited Granuloma Formation

Jeffrey H. Ruth,*, Nicholas W. Lukacs,† Kelly S. Warmington,*, Tim J. Polak,‡ Marie Burdick,‡ Steven L. Kunkel,† Robert M. Strieter,‡ and Stephen W. Chensue*§†

Eotaxin participation was analyzed during types 1 and 2 lung granuloma formation induced by embolizing Sepharose beads coupled to purified protein derivative (PPD) of Mycobacterium bovis or soluble Ags derived from Schistosoma mansoni eggs. Eotaxin was monitored by protein ELISA and semiquantitative reverse-transcriptase PCR mRNA analysis. Both types 1 and 2 granulomas released eotaxin, but levels were sixfold greater (on day 4) in the type 2 than for the type 1 or foreign body granulomas. Transcripts for eotaxin, IL-4, and CCR3 (eotaxin receptor) were also enhanced during type 2 granuloma formation. Anti-IL-4 treatment abrogated IFN-γ-producing cells in regional lymph nodes during the type 1 PPD response. Lymph nodes draining both types 1 and 2 lesions showed enhanced CCR3 mRNA, but this followed the time of maximum eotaxin protein and mRNA expression. Correlative, in vitro studies revealed that graded doses of eotaxin increased IFN-γ production from PPD-sensitive regional lymph node cultures, while monocyte-chemotactic protein-1, an important macrophage chemoattractant, had the opposite effect. These findings indicate that eotaxin expression is not limited to type 2 hypersensitivity granulomas, but also promotes IFN-γ production during mycobacterial responses.


Eotaxin (ETX), a potent eosinophil chemoattractant, was first described by Williams et al. using a model of allergic inflammation in the guinea pig, in which it was discovered that the fluid recovered by bronchoalveolar lavage induced the local accumulation of eosinophils when injected into the skin (1, 2). Subsequently it was discovered that mice and humans also express ETX (3, 4). Human eosinophils express receptors for ETX, which also recognize MCP-3 and RANTES, as indicated by binding studies (5). However, ETX does not appear to interact efficiently with the other known chemokine receptors. After local injection in vivo, ETX exclusively attracts eosinophils in guinea pigs, mice, and humans (2–6). In rodents, ETX expression was observed in models of allergic inflammation and conditions characterized by eosinophil accumulation (1). The potential role of ETX in allergic and eosinophilic inflammation was also suggested by Ponath et al. (5), who found expression of this chemokine in tissue cells (epithelium, fibroblasts, and smooth muscle cells) and immigrant leukocytes (including eosinophils) in the areas of eosinophil accumulation on immunohistochemically stained sections prepared from human allergic-type polyps (5). Thus, while there is considerable circumstantial evidence to support ETX as a critical eosinophil chemotaxin, few studies have attempted to determine its participation in other aspects of immunity.

To this end, we examined the participation of ETX in models of type 1 (eosinophil poor) and type 2 (eosinophil rich) cell-mediated lung granulomas in presensitized mice by embolization of Sepharose beads coupled to purified protein derivative (PPD) of Mycobacterium bovis or soluble Ags derived from Schistosoma mansoni eggs (SEA), respectively. We detected ETX production by protein ELISA and mRNA analysis by semiquantitative RT-PCR. Although both Th1- and Th2-generated pulmonary granulomas expressed ETX, levels were sixfold greater in the type 2 lesions than for the type 1 and control bead lesions. Accordingly, increased ETX and CCR3 mRNA expression was demonstrated in the type 2 lesions compared with the type 1 and control bead lesions. Furthermore, local ETX expression was clearly supported by IL-4 during type 2 granuloma formation since anti-IL-4 treatment in wild-type mice impaired ETX mRNA expression in granulomatous lungs.

Surprisingly, ETX depletion experiments showed that type 2 lesions were only modestly abrogated and type 1 lesions were similarly affected. This suggested that ETX was not critical to type 2 granuloma eosinophil recruitment and it potentially participated in the type 1 response. Further in vivo analysis revealed that in draining lymph nodes, ETX expression was greater during the type 1 response. Moreover, intrinsic ETX supported IFN-γ-producing cells in regional lymphoid tissue during the type 1, but not the type 2 response. These studies indicate ETX is not restricted to the eosinophil-rich type 2 granuloma, but has an expanded role in regulating T cell cytokine expression in lymphoid tissue.
Materials and Methods

Animals

Female CBA/J mice were obtained from The Jackson Laboratory (Bar Harbor, ME), maintained under specific pathogen-free conditions, and provided with food and water ad libitum.

Sensitization and granuloma induction

Types 1 and 2 granulomas were generated as previously described (7). Briefly, mice were sensitized by i.p. injection of 4 mg (weight) of live Bacillus-Calmette-Guerin (BCG) strain of M. bovis (Organon Technika, Durham, NC) or 3000 S. mansoni eggs suspended in 0.5 ml PBS. Fourteen to sixteen days later, BCG- and egg-sensitized mice were respectively challenged by i.v. with 6000 Sepharose 4B beads (in 0.5 ml PBS) covalently coupled with PPD of M. bovis kindly provided by Department of Agriculture Veterinary Division (Ames, IA) or SEA obtained from World Health Organization (Geneva, Switzerland).

Cytokines, Abs, and cytokine assays

All cytokines and chemokines used in this study were obtained as purified, carrier-free recombinant proteins from PeproTech (Rocky Hill, NJ) and R&D Systems (Minneapolis, MN). IL-2, IL-4, and IFN-γ were measured by ELISA using commercially available reagents (PharMingen, San Diego, CA); sensitivities ranged from 15 to 50 pg/ml. IL-12 was measured by sandwich ELISA, as previously described (8), with sensitivity to 50 pg/ml. Anti-murine ETX was prepared by multiple site immunization of rabbits by ELISA using commercially available reagents (PharMingen, San Diego, CA). The frozen tissues were suspended in Perfused lung lobes, excluding major bronchi, were snap frozen with liquid nitrogen. The mixture was then serially extracted with chloroform-phenol and chloroform-isomyl alcohol. The RNA was next precipitated at −70°C in ethyl alcohol, washed, and reprecipitated. The pellet was finally dissolved in diethyl-pyrocarbonate water, and RNA concentrations were determined spectrophotometrically before storage at −70°C. Yields are routinely >100 μg.

Primers and probes

Primers and probes (18–22 mer) were designed based upon nucleotide sequences downloaded from the NCBI database and using primer design software (Premier Biosoft International, Palo Alto, CA). Designed primer and probe sequences for each of the cytokines examined are as follows: Murine eotaxin, 5'-TTCTATTCTCTTCGTCAGCG-3', sense; 5'-AGGTTGTACGTCTGGTTGGTTG-3', antisense; 5'-CTCCATCCCACTTCCCTCTG-3', probe. Murine C-C chemokine receptor 3 (CCR3), 5'-TTCGACCGCAAACTGTGAC-3', sense; 5'-CTCTTGAGATCCGCAGACGAGCTG-3', antisense; 5'-CCTGCGAGCTGTCAGATG-3', probe. Murine IL-4, 5'-CTGACGCGCAAGAGATCTGTA-3', sense; 5'-TATGCAAGACACTTGGAAACGGC-3', antisense; 5'-GAGATCAGGCGGTTAGTTGTA-3', probe. Murine cyclophilin, 5'-ACCTAAAGGACTCAATCAGAACG-3', sense; 5'-GAGATCGCAGGCGGTTAGTTGTA-3', antisense; 5'-CATCGGTGACTCAAGGAGCTTC-3', probe.

Primers and probes were prepared by University of Michigan DNA Core Facility (Ann Arbor, MI). Probes were biotinylated with biotin-UTP using a standard 3'-end labeling kit (Boehringer Mannheim, Indianapolis, IN); unincorporated biotin was removed with QuickSpin columns (Boehringer Mannheim). Incorporation was confirmed by nick translation blotting, followed by streptavidin–alkaline phosphatase detection.

RT-PCR/ELISA detection of cytokine mRNA

The isolated RNA was first reverse transcribed to DNA as follows: To 20 μg of RNA (in 25 μl of diethyl-pyrocarbonate water) was added 3.6 μl of RNasin (Boehringer Mannheim) and 10 μl of random hexamer solution (500 mg/ml; Promega, Madison, WI), followed by heating to 70°C for 5 min in a thermocycler (Perkin-Elmer 9600; Perkin-Elmer, Norwalk, CT). The temperature was then reduced to 43°C; then 69 μl of a first strand buffer (Life Technologies, Grand Island, NY) containing dT, dNTPs, and 10 mCi [α-32P]dCTP. The reaction was then incubated for 2 h, then the reaction was stopped by heating to 70°C. The DNA was then subjected to PCR (14). Briefly, 5 μl of DNA was added to 95 μl PCR buffer containing unlabeled dNTPs (0.2 mM of each) plus digoxigenin-labeled dUTP, 1 μg sense primer, 1 μg antisense primer, and 5 U Taq polymerase (all from Boehringer Mannheim) in a thin-walled PCR tube. Amplification was then performed in a thermocycler as follows: 4 min at 95°C, followed by up to 23 cycles of 1 min at 95°C, 2 min at 57°C, and 1 min at 72°C. After cycling, there was a DNA extension period of 6 min at 72°C, then samples could be stored at −20°C before analysis.

Detection of PCR products was performed as follows: Initially, a series of amplification reactions using unlabeled dNTPs was performed, and the products were analyzed by agarose gel electrophoresis to confirm that primers yielded predicted products. Once confirmed, labeled products were generated and detected by PCR/ELISA (15). Briefly, 30 μl of amplified product was added to a sterile microfuge tube containing 40 μl of denaturing buffer (Boehringer Mannheim) and incubated 10 min. Next, 500 μl of hybridization buffer containing 4 ng/ml of appropriate (target gene) biotinylated probe. Negative controls included tubes with no DNA or DNA with inappropriate probe. The solution was mixed and 200 μl was distributed into duplicate wells of a multiwell, streptavidin-coated plate and incubated 3 h at 42°C. The plate was then washed and any bound product was detected with peroxidase-labeled anti-digoxigenin Ab by standard colorimetric reaction using ABTS substrate. A 96-well plate ELISA reader was used to measure OD at 405 nm. The OD is directly proportional to levels of target PCR product that is normalized to levels of a housekeeping gene, cyclophilin. This method is highly specific and has proven to be 10- to 100-fold more sensitive than gel detection.

Statistics

The Student’s t test was used to compare groups. Values of p > 0.05 were considered to indicate lack of significance.

Results

Eotaxin production is enhanced in type 2 granulomas

Mice were sensitized for 14 days with either BCG or 3000 schistosome eggs, then administered PPD or SEA Ag-coated beads to generate type 1 or type 2 hypersensitivity granulomas, respectively. Intact pulmonary granulomas were harvested at 1, 2, 4, and
8 days, then cultured for 24 h, and supernatants were assayed for spontaneous ETX expression. Control animals (unsensitized) received non-Ag-coated beads. As shown in Figure 1, enhanced ETX was observed in type 2 (SEA) granulomas at 4 and 8 days compared with PPD and CON bead lesions. Thus, ETX protein production was augmented during Th2-mediated inflammation.

Eotaxin receptor (CCR3) mRNA is enhanced during type 2 granuloma formation

To determine the potential local biologic effect of eotaxin, we compared mRNA expression for the eotaxin receptor, CCR3, in granulomatous lungs as normalized to cyclophilin expression. As shown in Figure 2, CCR3 mRNA was enhanced significantly in lungs with SEA lesions achieving levels twofold above normal on days 4 and 8. In contrast, the PPD and CON bead-challenged lungs remained near baseline. While the type 2 granuloma cell populations that express CCR3 are as yet undefined, these reportedly may include eosinophils and lymphocytes (16). Hence, these populations may potentially contribute to the local pool of CCR3 transcripts and respond to ETX.

Eotaxin mRNA is enhanced and expressed in parallel to IL-4 mRNA in type 2 granulomas and is impaired by anti-IL-4 treatment

Type 2 granulomas are characterized by IL-4 production that may support the enhanced local ETX synthesis. To test this possibility, we examined the relationship of ETX to IL-4. First, we analyzed granulomatous lungs at 1, 2, 4, and 8 days for ETX and IL-4 mRNA by RT-PCR. Levels were normalized to cyclophilin mRNAs, the chosen housekeeping gene, at the corresponding time points. Figure 3 (upper panel) shows the levels of ETX for PPD, SEA, and CON bead-challenged lungs. While eotaxin mRNA was increased above normal lung baseline (dashed line) for all groups, levels in SEA-bead lungs were threefold that of normal lung baseline and statistically above CON bead lungs on days 2, 4, and 8. This finding was in general agreement with our protein determinations. Figure 3 (lower panel) shows the corresponding IL-4 mRNA expression in these lungs. Paralleling the ETX message, IL-4 mRNA was enhanced significantly compared with CON lesions during the type 2 response on days 2, 4, and 8. This finding was fully consistent with previous studies showing that IL-4 contributes to local cell recruitment during the SEA response (9, 17, 18) and provided circumstantial support for reports that IL-4 promotes ETX expression (3). To directly test the role of IL-4 in eotaxin mRNA expression, we administered anti-IL-4 mAbs to sensitized wild-type mice that concurrently received SEA beads, then ETX mRNA was assessed at 2, 4, and 8 days (Fig. 4). Mice sacrificed on day 2 showed a
In vivo eotaxin depletion modestly abrogates both type 1 and type 2 pulmonary inflammation

To determine the contribution of ETX to the inflammatory responses, mice with either PPD or SEA lesions were administered Abs to ETX or nonimmune rabbit IgG. As shown in Figure 5, in vivo ETX depletion modestly, but significantly decreased the size of both types of lesions, suggesting that ETX contributed to both Th1- and Th2-mediated inflammation. Unexpectedly, differential analysis of dispersed granulomas revealed that ETX did not significantly contribute to local eosinophil recruitment (Table I), causing only a negligible decrease in the percentage of eosinophils in the type 2 lesion. The small abrogation of the PPD lesion was likewise surprising, as ETX was only marginally expressed locally in the type 1 granuloma; thus, other sites of potential ETX influence had to be considered.

Eotaxin expression is enhanced in regional lymphoid tissue during type 1-mediated inflammation

In view of a previous report demonstrating ETX mRNA in lymph nodes (19), we surmised that ETX may have a role in lymphoid tissue physiology. Initially, we assessed the relative expression of ETX in draining lymph nodes as well as the expression of its receptor, CCR3. Figure 6 shows spontaneous and Ag-elicited ETX production by draining lymph node cells of mice with PPD or SEA bead lesions as related to eotaxin and CCR3 mRNA expression. Spontaneously released ETX was increased significantly compared with controls on days 1 and 2 in the PPD, but only on day 1 in the SEA node cultures. This pattern was paralleled by the endogenous ETX mRNA expression in the snap-frozen tissue. As previously reported, there was detectable constitutive ETX mRNA in control nodes (dashed line) (19). Ag stimulation of cultures increased ETX levels throughout the 8-day study period for the PPD, but only on day 1 for the SEA response. Thus, ETX expression was heightened in lymph nodes draining lungs with type 1 granulomas and possibly contributed to regional lymphoid maturation events.

In the lungs, we analyzed lymph node CCR3 mRNA to reveal the presence of potential ETX-responsive populations (Fig. 6). Like ETX, there was baseline constitutive CCR3 mRNA expression (dashed line), but Ag bead-challenged mice showed patterns of enhancement. Specifically, SEA bead-challenged mice reached maximal expression on day 4, while PPD bead-challenged mice were comparatively delayed, reaching maximum on day 8. Thus, lymph nodes of both types 1 and 2 responses contained cells potentially responsive to ETX. Furthermore, the increase of CCR3 mRNA may reflect the generation of ETX-responsive populations.

Intrinsic eotaxin regulates IFN-γ production in lymphoid tissue

Since ETX was produced in lymphoid tissue, we examined the regional effects of in vivo ETX depletion on the draining lymph node cytokine profiles. The lymph nodes were teased into single cell suspensions and cultured with Ag, then assayed for cytokine production by ELISA. Lymph nodes of ETX-depleted, PPD bead-challenged mice showed significantly decreased levels of IFN-γ (by 85%), while IL-2 and IL-4 levels were unchanged (Table II). Thus, endogenous ETX promoted IFN-γ production during the PPD response. In the type 2 response, ETX depletion had no effect upon IL-2, IL-4, IL-5, and IL-12 levels, but ETX depletion tended to augment IFN-γ levels, suggesting a different immunoregulatory role for ETX during type 2 inflammation (Table II). This effect appeared to be restricted to the lymphoid tissues, since RT-PCR analysis of IFN-γ and IL-4 mRNA in granulomatous lungs showed no differences between control and anti-ETX-treated mice (data not shown).

Eotaxin promotes IFN-γ production by cultured type 1 draining lymph node cells

The above studies suggested that ETX was regulating IFN-γ-producing cells. This possibility was further tested in vitro. Types 1 and 2 pulmonary lesions were generated in mice; then on day 4, hilar lymph nodes were cultured with specific Ags and graded doses of either MCP-1 or ETX. Supernatants were collected and assayed for IFN-γ. As shown in Figure 7, ETX dose dependently increased IFN-γ levels in PPD lymph node cultures, while MCP-1 significantly decreased them. As previously reported, SEA lymph...
nodes produced low levels of IFN-γ (10). This degree of production was unaffected by MCP-1 or ETX treatment.

Discussion

This study is the first comprehensive investigation of the in vivo participation and cytokine-mediated regulation of ETX during pulmonary granulomatous inflammation. Other investigations have shown ETX to contribute to eosinophil accumulation in bronchoalveolar lavage fluid from allergen-challenged guinea pigs (2). Furthermore, aerosol exposure of naive guinea pigs to ETX caused a selective increase of eosinophils in bronchoalveolar lavage fluid (1). These studies provided evidence that ETX could potentially contribute to eosinophil accumulation in vivo. Clinically, ETX mRNA accumulates markedly in the lesions of patients with inflammatory bowel disease (ulcerative colitis and Crohn’s disease), but not in the lesions of patients with diverticulitis (4), providing circumstantial evidence for participation of ETX in clinical disease and in animal models of chronic inflammation. However, questions regarding the sources and the relative degree of ETX involvement remain largely unresolved. Therefore, this study was undertaken to elucidate the participation and contribution of ETX in models of chronic T cell-mediated hypersensitive inflammation.

Not surprisingly, our study showed the eosinophil-rich type 2 granuloma was associated with more ETX protein and mRNA than type 1 or non-T cell-mediated inflammation. In addition, mRNA for the eotaxin receptor, CCR3, was also increased during the type

<table>
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<th>Granuloma and Treatment</th>
<th>Lymphocytes</th>
<th>Large Mononuclears</th>
<th>Eosinophils</th>
<th>Neutrophils</th>
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</thead>
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<tr>
<td>PPD (type 1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control IgG</td>
<td>56 ± 1</td>
<td>36 ± 1</td>
<td>1 ± 0.1</td>
<td>7 ± 0.3</td>
</tr>
<tr>
<td>Anti-ETX</td>
<td>56 ± 5</td>
<td>39 ± 8</td>
<td>0</td>
<td>7 ± 1.4</td>
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<tr>
<td>SEA (type 2)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control IgG</td>
<td>49 ± 2</td>
<td>30 ± 1.4</td>
<td>18 ± 0.2</td>
<td>2 ± 3</td>
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<tr>
<td>Anti-ETX</td>
<td>47 ± 2</td>
<td>34 ± 0.3</td>
<td>16 ± 1.4</td>
<td>2 ± 1</td>
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Table I. Effect of in vivo ETX depletion on cell composition of dispersed type 1 and type 2 granulomas.

*Values are mean ± SD of two separate experiments; six to eight mice per group. Levels are shown for Ag-stimulated cultures. Mice with type 1 or type 2 pulmonary granuloma formation were treated with 5 mg of anti-ETX or control IgG as described. The pulmonary granulomas were removed and digested, and duplicate cytospin preparations were made to determine the cell composition by a 200 cell differential on day 4.

**FIGURE 6.** Eotaxin protein in draining lymph node cultures during type 1 and type 2 granuloma formation, as related to eotaxin and CCR3 mRNA expression. Draining mediastinal lymph nodes were collected from sensitized mice with developing type 1 or type 2 lesions and were cultured or snap frozen. Upper left panel, Spontaneous eotaxin production in 24-h culture supernatants. Right upper panel, Ag-elicited eotaxin. Lower left panel, Eotaxin mRNA expression. Lower right panel, CCR3 mRNA expression. Dashed lines show levels in lymph nodes from control bead-challenged mice. The latter and day 0 nodes were not significantly different from normal controls and showed no significant changes over the 8-day study period. Bars represent mean ± SEM of two separate experiments with six to eight mice per group. *, p < 0.05 as compared with control nodes.
2 response. This finding suggested that local ETX expression was up-regulated by Th2-related cytokines. This impression was supported by time-course data showing parallel ETX and IL-4 mRNA expression as well as diminished ETX mRNA expression in anti-IL-4-treated and IL-4 knockout mice (7). Thus, we demonstrated that IL-4 was most likely an important inducer of ETX. These findings fully agree with those of Rothenberg et al., who identified a positive regulatory role for IL-4 in ETX expression using IL-4-transfected tumor cells in vivo (3).

In view of the strong circumstantial relationship of ETX to the type 2 lesion, it was surprising that in vivo ETX depletion had marginal to negligible effect on the size and eosinophil content of SEA lesions. However, this result is consistent with the recent report of Rothenberg et al., showing that disruption of the ETX gene failed to impair late phase eosinophil accumulation in a keratitis model (19). It is possible that the lack of an effect on the type 2 lesions was due to incomplete ETX neutralization, but we have similarly shown that ETX expression is profoundly impaired in IL-4 knockout mice despite strong eosinophil recruitment (7). Likewise, preliminary studies in our laboratory indicate that eosinophil recruitment to type 2 granulomas is unimpaired in ETX knockout mice. A more reasonable explanation is that redundant chemotactic activities are present and ETX is just one of several agents that may recruit eosinophils.

Further surprising results arose from in vivo ETX depletion experiments showing that PPD lesions were modestly reduced by anti-ETX. This was unexpected, as these granulomas have low numbers of eosinophils and weak IL-4 expression. This observation led to our investigation of other roles for ETX in types 1 and 2 inflammation. Eotaxin mRNA is known to be constitutively expressed, consistent with our previously published finding that MCP-1 inhibits IL-12 production (20). Thus, ETX can be included among other chemotactic proteins (e.g., macrophage-inflammatory protein-1α) that have immunoregulatory properties (21–23). Our findings suggest that ETX plays a role in the expression of Th1 cells in lymphoid tissues distal from the inflammatory response. It also explains the modest abrogating effect of anti-ETX on PPD lesions, as they are in part IFN-γ dependent.

Table II. Effect of in vivo ETX depletion on draining lymph node cytokine profiles during type 1 and type 2 granuloma formation

<table>
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<tr>
<th>Granuloma and Treatment</th>
<th>Cytokine (ng/ml)</th>
<th>IL-2</th>
<th>IL-12</th>
<th>IFN-γ</th>
<th>IL-4</th>
<th>IL-5</th>
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<td>IgG</td>
<td>0.24 ± 0.08</td>
<td>1.1 ± 0.12</td>
<td>3.90 ± 1.19</td>
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<td>Anti-ETX</td>
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<td>1.2 ± 0.09</td>
<td>0.57 ± 0.16*</td>
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<tr>
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<td>IgG</td>
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</table>

*Values are mean ± SEM of two separate experiments; six to eight mice per group. Levels are shown for Ag-stimulated IgG; next the lymph nodes draining the lesions were assessed for cytokine profiles on day 4.

In Figure 7, in vitro effect of eotaxin or monocyte-chemotactic protein-1 treatment on IFN-γ production by type 1 (PPD) and type 2 (SEA) cultured lymph node cells. Lymph node cells draining day 4, type 1 or type 2 granulomatous lungs were cultured with 5 μg/ml of specific Ag (PPD or SEA) and graded concentrations of ETX or MCP-1 for 24 h, then supernatants were collected and assayed for IFN-γ production by ELISA. Bars represent mean ± SEM of three separate experiments with a total of 9 to 12 mice per group. *, p < 0.05 as compared with Ag-stimulated, chemokine-untreated cultures.

FIGURE 7. In vitro effect of eotaxin or monocyte-chemotactic protein-1 treatment on IFN-γ production by type 1 (PPD) and type 2 (SEA) cultured lymph node cells. Lymph node cells draining day 4, type 1 or type 2 granulomatous lungs were cultured with 5 μg/ml of specific Ag (PPD or SEA) and graded concentrations of ETX or MCP-1 for 24 h, then supernatants were collected and assayed for IFN-γ production by ELISA. Bars represent mean ± SEM of three separate experiments with a total of 9 to 12 mice per group. *, p < 0.05 as compared with Ag-stimulated, chemokine-untreated cultures.
Interestingly, while ETX was shown to promote IFN-γ during the type 1 response, it tended to inhibit IFN-γ production in lymph nodes draining type 2 inflammation. These data suggest differential responsiveness of lymphoid tissue depending upon whether committed to a type 1 or 2 inflammatory response. Therefore, if ETX promotes IFN-γ production by type 1 T cells, it would help sustain the type 1 environment by down-regulating Th2 cell activity through cross-regulatory mechanisms (25). Conversely, during type 2 inflammation, ETX appeared to impair IFN-γ production. However, anti-ETX-mediated augmentation of IFN-γ did not appear to cause cross-regulatory impairment of type 2 cytokine production, probably because the increase was insufficient to affect the response, especially as this response is already subject to endogenous IFN-γ-mediated regulation (24). The mechanisms behind the differential effect of ETX on types 1 and 2 responses are unclear, and we hope our ongoing studies of ETX knockout mice will offer further insights. One possibility is that ETX receptors transduce opposing signals in type 1 and type 2 committed T cell populations.

Eotaxin is reported to exert its effects via the CCR3 G protein-coupled receptor (26, 27). Recently, this receptor has been reported to have selective expression on human Th2 cells (28). In accord with this notion, we observed greater CCR3 expression in type 2 granulomas. However, our findings suggest the existence of more complex relationships within lymphoid tissues in which there appears to be a dynamic expression of chemokines and chemokine receptors that may be involved in the regulation of both Th1 and Th2 cell maturation and migration. Our study suggests an expanded role for ETX, not only as a potential chemotactic factor, but also as an IFN-γ-promoting molecule in regional lymph nodes during the type 1 mycobacterial response. Therefore, ETX may take part in protecting the host against intracellular infections or cellular growth abnormalities. Thus, as a functional molecule, ETX may not be restricted to eosinophil-dominant type 2 responses.

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