IL-12 Produced by Dendritic Cells Augments CD8+ T Cell Activation through the Production of the Chemokines CCL1 and CCL17


http://www.jimmunol.org/content/181/12/8576

**Why *The JI***?
- **Rapid Reviews! 30 days** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Speedy Publication!** 4 weeks from acceptance to publication

*average

**References**  This article cites 75 articles, 36 of which you can access for free at: http://www.jimmunol.org/content/181/12/8576.full#ref-list-1

**Subscription**  Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

**Permissions**  Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**  Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
IL-12 Produced by Dendritic Cells Augments CD8+ T Cell Activation through the Production of the Chemokines CCL1 and CCL17


IL-12 family members are an important link between innate and adaptive immunity. IL-12 drives Th1 responses by augmenting IFN-γ production, which is key for clearance of intracellular pathogens. IL-23 promotes the development of IL-17-producing CD4+ T cells that participate in the control of extracellular pathogens and the induction of autoimmunity. However, recent studies have shown that these cytokines can modulate lymphocyte migration and cellular interactions. Therefore, we sought to determine the individual roles of IL-12 and IL-23 in naive CD8+ T cell activation by addressing their ability to influence IFN-γ production and cellular interaction dynamics during priming by *Listeria monocytogenes*-infected dendritic cells (DC). We found that IL-12 was the major cytokine influencing the level of IFN-γ production by CD8+ T cells while IL-23 had little effect on this response. In addition, we observed that IL-12 promoted longer duration conjugation events between CD8+ T cells and DC. This enhanced cognate interaction time correlated with increased production of the chemokines CCL1 and CCL17 by WT but not IL-12-deficient DC. Neutralization of both chemokines resulted in reduced interaction time and IFN-γ production, demonstrating their importance in priming naive CD8+ T cells. Our study demonstrates a novel mechanism through which IL-12 augments naive CD8+ T cell activation by facilitating chemokine production, thus promoting more stable cognate interactions during priming. The Journal of Immunology, 2008, 181: 8576–8584.

Department of Microbiology and Immunology, Wake Forest University School of Medicine, Winston-Salem, NC 27157

Received for publication March 5, 2008. Accepted for publication October 13, 2008.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This research was supported by the National Institutes of Health Research Project Grant Program (R01) Grant AR057770-01A1 (to E.M.H.). Additional support was provided by the National Institutes of Health Predoctoral Fellowship Award for Minority Students (F31) Grant AT73245-01A1 (to C.J.H.).

2 Address correspondence and reprint requests to Dr. Elizabeth M. Hiltbold, Department of Microbiology and Immunology, 5109 Gray Building, Wake Forest University School of Medicine, Winston-Salem, NC 27157. E-mail address: bhiltbol@wfubmc.edu

3 Abbreviations used in this paper: DC, dendritic cell; Lm, *Listeria monocytogenes*; WT, wild type; MFI, mean fluorescence intensity; ICS, intracellular cytokine staining.

Copyright © 2008 by The American Association of Immunologists, Inc. 0022-1767/08/$2.00
CD4+ T cells (driving a Th1-type response) as well as NK and CD8+ T cells. IL-12 has been shown to increase CD8+ T cell cytolyis, survival, proliferation, and influence the ability of these lymphocytes to migrate to inflammatory foci. A more recently recognized activity of IL-23 is the ability to stimulate the production of IL-17 by CD4+ T cells. These Th17 cells are critical for the development of autoimmune diseases and immunity to certain extracellular pathogens (24–29, 37–45). However, the role of IL-23 in the activation of naive CD8+ T cells is still being investigated.

Since IL-12 and IL-23 have very potent effects on specific immune responses, we wanted to determine the individual contributions of IL-12 and IL-23 on CD8+ T cell priming by DC. In addition, we wanted to identify the mechanism(s) through which these cytokines modulated naive CD8+ T cell activation. To approach these goals, we used DC generated from mice lacking both IL-12 and IL-23 (p40+/−) or only IL-12 (p35+/−). We found that IL-12, but not IL-23, augmented IFN-γ production by CD8+ T cells. We also found that IL-12 promoted longer duration interactions between CD8+ T cells and Listeria-infected DC. To address the potential mechanisms through which IL-12 regulated T cell-DC interactions, we examined chemokine production in the presence or absence of IL-12. The production of CCL1 and CCL17 was increased by DC in the presence of IL-12. Further studies determined that these chemokines directly increased the duration of conjugation as well as IFN-γ production by CD8+ T cells.

Materials and Methods

**Mice**

C57BL/6, IL-12p35−/−BL/6-IL12a−/−/BL-6-IL12b−/−/B6.129SIIIl12r2−/−/H11001, OT-I, and OT-II TCR-transgenic mice specific for OVAa3,−25, B2K-presented by OT-I T, and OVAa3,−33 presented by I-A^a were purchased from The Jackson Laboratory. P14 TCR-transgenic mice specific for the lymphocytic choriomeningitis virus gp peptide 33–41 were provided by Dr. J. M. Grayson (Wake Forest University School of Medicine, Winston-Salem, NC). All mice were maintained and bred in the animal facility at Wake Forest University School of Medicine.

**Antibodies**

Neutralizing Abs. To neutralize the activity of IL-12 and IL-23, anti-p40 (clone C15.6; BioSource International) or just anti-p19 (clone G23-8; eBioscience) neutralizing Abs (10 μg/ml) were added before T cell addition and maintained throughout the assay, unless otherwise indicated. Neutralizing Abs against the chemokines TARC/CCL17 (clone 119004; R&D Systems) and TCA-3/CCL1 (catalog no. AF845; R&D Systems) were added at 10 μg/ml before T cell addition and maintained throughout the assay.

**Surface and intracellular cytokine staining (ICS) Abs.** Fluorescent Abs measuring the expression of the mouse DC costimulatory molecules CD40 (clone 3D23), CD80 (clone 16-10A1), and CD86 (GL1) and the phenotypic marker CD11c were purchased from BD Biosciences. IFN-γ production by CD8+ OT-I T cells was measured using fluorescent Abs to CD8 (clone 53-6.7; BD Pharmingen) and IFN-γ APC (clone XMG1.2; BD Pharmingen).

**DC propagation**

Bone marrow-derived DC were generated as previously described (14). Briefly, bone marrow was removed from the tibiae and femurs of 8- to 10-wk-old C57BL/6 (or strain indicated) mice. RBC were lysed and the progenitor cells (5 × 107/ml) were resuspended and plated in RPMI 1640 containing 10% FCS supplemented with 10 ng/ml GM-CSF (generated from a recombinant baculovirus expression system). DC were cultured for 6 days at 37°C in 5% CO2 and given fresh medium and cytokine on days 2 and 4. DC used for the described experiments were between 90 and 95% CD11c+ and expressed low levels of CD40, CD80, and CD86, which are characteristic of immature DC (data not shown).

**In vivo infection of mice**

C57BL/6 (wild type (WT)) or IL-12-deficient (p35−/−) DC were mock treated (PBS only) or infected with 1 LD50 of Lm strain 10403S delivered i.v. (5 × 108). At 18 h after infection, mice were sacrificed and inguinal, lumbar, and mesenteric lymph nodes as well as spleens were harvested. RNA was isolated for chemokine analysis from DC following enrichment for CD11c+ cells.

**In vitro infection of DC**

For T cell priming assays, DC were seeded at 2 × 105/well and infected with WT Listeria (strain 10403S) at a multiplicity of infection (MOI) of 1. Four hours after infection, OVA peptidepeptide257–264 was added at a concentration of 0.1 ng/ml (or as indicated) with chloramphenicol (10 μg/ml) and gentamicin (10 μg/ml). Twenty-four hours after infection, OT-I T cells were added at 10:1 T:DC. For some experiments, OT-I T cells were stained with CFSE before culture with DC.

**Time-lapse video microscopy**

DC were prepared and infected as in T cell priming assays with the following modifications: Day 6 WT, IL-12p55−/−, or IL-12p40−/− DC (105) were seeded in T25 flask and infected with Lm at a MOI of 1. After 4 h, antibiotics were added and the flask was at a ratio of 10:1 T cells:DC. For neutralization experiments, isotype control protein or neutralizing Abs to CCL1, CCL17, or IL-12/23 p40 were added to the flask at a concentration of 0.1 μg/ml. At 24 h after infection, OT-I T cells were added to the flask at a ratio of 10:1 T cells:DC. For neutralization experiments, isotype control protein or neutralizing Abs to CCL1, CCL17, or IL-12/23 p40 were added to the flask at a concentration of 0.1 μg/ml. The total time of conjugation between individual CD8+ T cells and DC was determined by measuring the ligation (on) and detachment (off) times of a T cell with DC conjugate. At least 50 interactions per experiment were monitored. Interaction times are expressed as dissociation curves between T cell and DC with the percentage of T cells in conjugate vs total time of conjugation with DC (in min). A more gradual slope indicates longer duration interactions between a population of T cells and DC. Time-lapse phase-contrast images were recorded with an exposure time of 5–5 s frame intervals using an Olympus IX70 enclosed camera with an incubation chamber set at 37°C. Video recordings were captured over a 48-h period.

**ELISAs**

Culture supernatants from either DC infection/mature assay or T cell-priming assays were collected at the indicated times. These supernatants were then probed for the presence of the following cytokines using ELISA kits according to the manufacturer’s instructions: T cell activating gene 3 (TCA3; CCL1) and thymus and activation-regulated chemokine (CCL17) (Duoset ELISA; R&D Systems), IL-12p40 (OptEIA kit; BD Biosciences), IL-23 (eBioscience), and IFN-γ (OptEIA kit; BD Biosciences).

**Cytokine/chemokine protein array**

WT, IL-12p55−/−, and IL-12p40−/− were infected with Lm at a MOI of 1. Twenty-four hours after infection, supernatants were harvested and probed for various cytokines and chemokines according to the manufacturer’s instructions (Raybiotech Mouse Cytokine Ab Array 3).

**Real-time RT-PCR**

Real-time PCR was performed according to the manufacturer’s instructions (Applied Biosystems). Briefly, RNA was isolated from bone marrow-derived DC or DC enriched from the spleens and lymph nodes of Lm-infected mice using the RNAqueous kit (Ambion). Once isolated, 20 ng of RNA of each sample was used in each reaction. Primer and probe sets were used to detect CCL1, CCL17, and ArcB messages were purchased from Applied Biosystems. TaqMan Universal PCR Master Mix was purchased from Applied Biosystems and nuclease-free water was purchased from Ambion. The reverse transcriptase used in these studies was Moloney murine leukemia virus-reverse transcriptase, which was purchased from Invitrogen. The reactions were performed and analyzed using the Applied Biosystems Prism 7000 Sequence Detection System.

**Column enrichment of ex vivo DC**

DC were enriched (CD11c+ selection) from the spleens and lymph nodes of Lm-infected mice according to the manufacturer’s instructions (Miltenyi Biotec). LS columns were used for the spleen and MS columns were used for the lymph nodes.

**Results**

IL-12, but not IL-23 significantly augments IFN-γ production by CD8+ T cells

Previous work from our laboratory and others has shown that IL-12 production increases IFN-γ production by CD8+ T cells (3,
FIGURE 1. IL-12, but not IL-23 augments IFN-γ production by naive CD8⁺ T cells. A. CFSE-stained OT-I T cells were primed by Listeria-infected OVA-pulsed WT, p35⁻/⁻, or p40⁻/⁻ DC and IFN-γ production was determined by ICS on day 3 of priming. IFN-γ MFI of T cells is denoted in the upper left corner. Contour plots are representative of 10 independent experiments. B. The combined data from eight independent flow cytometric experiments are graphed. The solid line indicates the amount of IFN-γ T cells produced when primed in the absence of OVA peptide. The mean ± SD is shown. C, IFN-γ production by CD8⁺ T cells during priming measured by ELISA. CD8⁺ T cells were primed by either WT, p35⁻/⁻, or p40⁻/⁻ DC and the amount of IFN-γ that accumulated over 3 days was determined via ELISAs. The average concentration of IFN-γ from three independent experiments is graphed. D. CFSE-stained OT-I T cells were primed by Listeria-infected OVA-pulsed DC in the presence of neutralizing Abs against IL-23 (anti-p19) or IL-12/23 (anti-p40) and IFN-γ production was determined by ICS on day 3 of priming. Contour plots are representative of eight independent experiments. E. The combined data from 10 independent experiments are graphed. The solid line indicates the amount of IFN-γ T cells produced when primed by WT DC in the absence of OVA peptide. The mean ± SD is shown. Significance compared with the rat Ig or WT DC controls was determined using Student’s t test with *, p < 0.05; **, p < 0.01; and ***, p < 0.001.

14, 28, 29, 46–48). In addition, it has been reported that IL-12 augments the proliferative capacity of these cells (3, 18, 19). These conclusions were drawn primarily from neutralization studies in which the p40 subunit of the cytokine was targeted; therefore, the individual contributions of IL-12 or IL-23 to these responses could not be distinguished. To address the relative contributions of IL-12 and IL-23 to CD8⁺ T cell activation, we primed naive CFSE-stained OT-I T cells with either WT, p35⁻/⁻ (no IL-12), or p40⁻/⁻ (no IL-12 or IL-23) DC. Before incubation with T cells, DC were infected with Listeria to induce cytokine production and were pulsed with OVA peptide. Infection of DC with Listeria resulted in low levels of IL-23 production from WT (53 pg/ml) and p35⁻/⁻ DC (44 pg/ml), but not from p40⁻/⁻ DC (7 pg/ml vs 10 pg/ml produced by uninfected DC; data not shown).

On day 3 of priming, CD8⁺ T cell IFN-γ production and proliferation were measured (Fig. 1A). Proliferation of CD8⁺ T cells was not influenced by IL-12 or IL-23. However, in the absence of IL-12, we observed a significantly decreased level of IFN-γ produced on a per cell basis (2-fold reduction in mean fluorescence intensity (MFI)). In the absence of both IL-12 and IL-23 (p40⁻/⁻), we observed the most pronounced decrease in the IFN-γ MFI; yet, it was not significantly less than that observed in the absence of IL-12 (p35⁻/⁻) alone (Fig. 1B). In addition, the amount of IFN-γ secreted by CD8⁺ T cells over the 3-day priming period was reduced 3-fold compared with WT in the absence of IL-12 (Fig. 1C).

Again, we observed no significant difference between the p35- and p40-deficient DC in their ability to influence IFN-γ secretion over this period.

To more specifically address the role of IL-23, this cytokine was neutralized using an anti-p19 Ab. IL-23 neutralization did not significantly decrease OT-I IFN-γ production on a per cell basis when primed by WT DC (control IFN-γ MFI, 262 and anti-p19 IFN-γ, MFI 271; Fig. 1D). In contrast, when both IL-12 and IL-23 were neutralized (anti-p40), IFN-γ production by OT-I was significantly decreased (control IFN-γ MFI, 262 and anti-p40 IFN-γ MFI, 74.6). Neutralization of IL-23 alone or IL-12 and IL-23 in combination did not alter proliferation (Fig. 1D). In addition, these results suggest that IL-23 does not augment IFN-γ production by CD8⁺ T cells and confirm that IL-12 is a critical mediator of this process.

We next wanted to address the possibility that these observations could be attributed to changes in costimulatory molecule expression or Ag-presenting capacity of DC in the absence of IL-12 or IL-12/23. Therefore, we measured the up-regulation of costimulatory molecules (CD86, CD80, and CD40) and the Ag
IL-12 promotes long duration interactions between CD8+ T cells and Lm-infected DC

Several studies have monitored the dynamics of interactions between T cells and APCs to determine how these interactions impact T cell proliferation and function (21, 49–51). What has become clear is that full activation of naive CD8+ T cell requires long duration interactions with DC (21). Because IL-12 production peaks within 12 h of listerial infection and is known to influence lymphocyte migration during inflammation (52, 53), we decided to test the effect of IL-12 on the duration of interactions between CD8+ T cells and DC. DC were infected in a flask for 4 h, at which time OVA peptide and antibiotics were added. Twenty hours postinfection, anti-p40-neutralizing Abs and OT-I T cells were added to the culture. Time-lapse video microscopy was then performed over a 48-h period and the on/off times of individual T cell-DC interactions were measured. WT DC promoted long duration interactions with CD8+ T cells with 50% of the interactions lasting 127 min (Fig. 2A). Only transient interactions occurred in the absence of Ag. Neutralization of the p40 subunit of IL-12 and IL-23 significantly reduced the conjugation time between CD8+ T cells and DC with 50% of the interactions lasting only 27 min (Fig. 2A).

To determine the relative contributions of IL-12 and IL-23 to this interaction, CD8+ T cells were primed by WT, p35−/−, or p40−/− DC and the duration of conjugation was determined as described above. In this study, we also observed that in the absence of IL-12 and IL-23 (p40−/−), the majority of interactions were of short duration, with 50% of the interactions lasting only 16 min (Fig. 2B). Again, there was no significant difference in the duration of interaction in the absence of IL-12 (p35−/−) vs both IL-12 and IL-23 (p40−/−). This observation indicated that IL-12 could promote long duration interactions between CD8+ T cells and DC and that IL-23 did not significantly impact this interaction.

IL-12 augments CCL1 and CCL17 production by Lm-infected DC

IL-12 is not widely regarded as a chemotactic mediator, yet we observed enhanced cell-cell interaction when it was present. Therefore, we decided to test whether IL-12 affected the expression of chemokines by DC. To identify candidate chemokines produced by DC in an IL-12-dependent manner, we used a cytokine/chemokine protein array. WT, p35−/−, and p40−/− DC were infected with Lm at a MOI of 1 and, 24 h after infection, the culture supernatants were subjected to this multiplex analysis. Interestingly, expression of the two chemokines, CCL1 and CCL17, was markedly reduced in the absence of IL-12 as well as IL-12/23 (data not shown). However, the lack of IL-12 and IL-23 did not appear to significantly alter the production of other chemokines as strongly, including MCP-1, MIP-1α, MIP-1γ, or RANTES (data not shown).

We next wanted to more quantitatively measure the induction of CCL1 and CCL17 by DC in the presence or absence of IL-12 or IL-23. As a first step, RNA was isolated from either WT, p35−/−, p40−/−, or DC lacking the IL-12R (all 4-fold reduced) compared with the levels generated in Lm-infected DC. Real-time RT-PCR analysis was performed to measure these chemokine messages. We found that the CCL1 message was significantly reduced in the absence of IL-12, IL-12/23, and the IL-12R (all 4-fold reduced) compared with the levels found in WT DC (Fig. 3A). We also observed reduced CCL17 message in the absence of IL-12, IL-12/23, and the IL-12R (8-fold reduced) compared with the levels generated in Lm-infected WT DC (Fig. 3A). Taken together, these results indicate that IL-12 (but not IL-23) enhances the production of CCL1 and CCL17 message by Lm-infected DC and that this enhancement is dependent on signaling through the IL-12 receptor.

To more directly determine the role of IL-12 in augmenting CCL1 and CCL17 protein production, ELISA analysis was performed on the supernatants of WT or p35−/− DC infected with Listeria at 24 h after infection. We found that p35−/− DC produced significantly less CCL17 (3-fold reduction) at 24 h after infection than WT DC (Fig. 3B). The amount of CCL1 produced by p35−/− DC was also reduced by 2-fold compared with WT DC (Fig. 3C); however, CCL1 secretion was very modest overall. Additionally, we found significant reductions in secreted CCL17 (3-fold) and CCL1 (>2-fold) if DC lacked expression of the IL-12
Physical interaction between T cells and DC, time-lapse video microscopy was used to measure the duration of interaction between T cells and DC in the presence of neutralizing Abs against CCL1 and CCL17. The neutralization of these chemokines resulted in a significant decrease in the duration of conjugation between the OT-I CD8\(^+\) T cells and DC compared with control samples (Fig. 4A). To determine whether this phenomenon could be generalized to other CD8\(^+\) T cells, not just OT-I, we included an additional source of naive CD8\(^+\) T cells, the P14 TCR-transgenic cells, specific for the lymphocytic choriomeningitis virus peptide gp\(_{33-41}\) presented by H-2D\(^b\). As in the case of the OT-I, we also observed a significant decrease in the duration of interaction of the P14 T cells with DC in the absence of CCL1 and CCL17 (Fig. 4B). Thus, the diminished T cell-DC interaction time observed in the absence of IL-12 seems to correlate with similar decreases in interaction times observed when these two chemokines are neutralized.

Neutralization of CCL1 and CCL17 significantly reduces the amount of IFN-\(\gamma\) produced by CD8\(^+\) T cells

Based on the observation that IL-12 augmented the production of the chemokines CCL1 and CCL17 and that neutralization of these chemokines reduces T cell-DC interaction time, we decided to determine whether the neutralization of these chemokines also affected IFN-\(\gamma\) production by CD8\(^+\) T cells. OT-I and P14 CD8\(^+\) T cells were primed by DC in the presence of the indicated neutralizing Abs (Fig. 4, C and D). The neutralization of CCL1 or CCL17 alone resulted in only a modest (not statistically significant) reduction in the amount of IFN-\(\gamma\) produced on a per cell basis by OT-I and P14 T cells. However, the neutralization of CCL1 and CCL17 in combination did significantly reduce IFN-\(\gamma\) production by both T cell populations (2-fold less than the control). These observations indicate that CCL1 and CCL17 together augment IFN-\(\gamma\) production by CD8\(^+\) T cells but that neither one alone significantly impacts this response.

Neutralization of CCL1 and CCL17 does not reduce IFN-\(\gamma\) by CD4\(^+\) T cells

To determine whether CCL1 and CCL17 also augmented IFN-\(\gamma\) production by CD4\(^+\) T cells, OVA-specific, OT-II-transgenic CD4\(^+\) T cells were primed by Lm-infected DC in presence of neutralizing Abs against CCL1, CCL17, or both. Parallel experiments with OT-I T cells were used as positive controls. On day 3 of priming, IFN-\(\gamma\) production was measured by ELISA. We found that neutralization of IL-12/23 (anti-p40) significantly reduced IFN-\(\gamma\) production by both OT-I and OT-II T cells (Fig. 5). IFN-\(\gamma\) production by CD8\(^+\) T cells was also significantly reduced when CCL1 and CCL17 were neutralized in combination. However, the single or double neutralization of these chemokines did not significantly alter IFN-\(\gamma\) production by CD4\(^+\) T cells. These results indicate that unlike CD8\(^+\) T cells, CD4\(^+\) T cell production of IFN-\(\gamma\) is not augmented by CCL1 and CCL17.

IL-12 augments CCL1 and CCL17 production from DC induced by listerial infection in vivo

Having determined that CCL1 and CCL17 production was enhanced by IL-12 in vitro, we wanted to determine whether this enhancement was also observed in vivo. To address this question, WT or IL-12-deficient (p35\(^{-/-}\)) mice were i.v. infected with Lm (1 LD\(_{50}\)). At 18 h after infection lymph nodes (pooled mesenteric, inguinal, and lumbar) were harvested. DC were enriched from these organs using CD11c\(^+\) selection (cells were >85% CD11c\(^+\)). Once isolated, we purified RNA and determined the message levels for CCL1 and CCL17 using real-time PCR.

We observed increased message levels for CCL1 and CCL17 in DC isolated from Lm-infected WT and p35\(^{-/-}\) mice compared with mock-treated mice (Fig. 6). As a normalization control, actin...
message levels were measured and were found to be equivalent in DC isolated from infected WT and p35/H11002/mice (data not shown). In Lm-infected mice, we observed a greater than 6-fold decrease in the message levels for CCL17 and a 4-fold decrease in CCL1 levels in DC isolated from the lymph nodes of IL-12-deficient mice compared with WT mice (Fig. 6). These data indicate that CCL1 and CCL17 production in response to listerial infection in

**FIGURE 4.** CCL1 and CCL17 augment T cell-DC interaction time and IFN-γ production by CD8⁺ T cells. A and B. OT-I and P14 were primed by OVA or gp33–41-pulsed *Listeria*-infected DC for 72 h in vitro with control or anti-CCL1/CCL17-neutralizing Abs. The duration of interaction between T cells and DC was determined via time-lapse video microscopy. Dissociation curves are combined data from at least three independent experiments per condition. The solid line represents 50% of the interactions analyzed. Significance compared with the control samples was determined by Cox proportional hazard regression analysis. The neutralization of CCL1/17 resulted in a significant decrease in the duration of interaction of OT-I (p < 0.01) and P14 (p < 0.01) with DC compared with controls. C. OT-I and P14 T cells were primed by *Listeria*-infected, OVA, or gp33–41 peptide-pulsed DC for 72 h in vitro. Control, anti-CCL1, anti-CCL17, or a combination of anti-CCL1/17-neutralizing Abs was added when T cells were added with DC. D. The IFN-γ MFI of T cells was determined at 72 h via ICS staining. Compiled data represent eight independent experiments for the OT-I T cells and four independent experiments for the P14 T cells. The relative statistical significance of either OT-I or P14 CD8⁺ T cell responses was determined compared with the same T cell in the absence of neutralization using Student’s t test with *, p < 0.05; **, p < 0.01; and ***, p < 0.001.

**FIGURE 5.** CCL1 and CCL17 do not augment IFN-γ production by CD4⁺ T cells. OT-I and OT-II T cells were primed by Lm-infected DC in the presence or absence of anti-p40, anti-CCL1, anti-CCL17, or a combination of anti-CCL1/17-neutralizing Abs in vitro for 3 days. On day 3 of priming, supernatants were filtered and assayed for the concentration of IFN-γ via ELISAs. Histograms represent combined data from five independent experiments for OT-I (A) and three independent experiments for OT-II T cells (B). Significance of neutralized samples was calculated in comparison to control OT-I CD8⁺ T cells or OT-II CD4⁺ T cells without neutralization using Student’s t test with *, p < 0.05; **, p < 0.01; and ***, p < 0.001.

**FIGURE 6.** IL-12 augments CCL1 and CCL17 messages in DC during Lm infection in vivo. WT and IL-12-deficient (p35/H11002/) DC were mock treated (PBS) or infected with 1 LD50 of Lm i.v. At 18 h after infection, RNA was isolated from DC enriched from the lymph nodes of treated mice. Four mice per group were analyzed and data represent the mean ± SD. CCL1 and CCL17 message levels were determined via real-time PCR. Significance was assessed compared with DC isolated from WT mice using Student’s t test for each gene with *, p < 0.05; **, p < 0.01; and ***, p < 0.001.
vivo is enhanced by IL-12 to at least the same extent as that observed in vitro.

**Discussion**

The ability of IL-12 to promote strong Th1 responses by enhancing IFN-γ production from various lymphocytes is well documented (25, 27-29, 38). However, its ability to influence other parameters of T cell activation, such as the physical interactions between immune cells during priming, has not been well characterized. To our knowledge, this is the first report which shows that IL-12 influences the interactions between CD8⁺ T cells and DC during priming and points to a mechanism through which this effect is mediated.

In our study, we found that IL-12 produced by DC augmented the production of the chemokines CCL1 and CCL17. These chemokines were found to promote longer duration interactions between CD8⁺ T cells and *Listeria*-infected DC. These longer duration interactions correlated with increased IFN-γ production by CD8⁺ T cells. Finally, neutralization of CCL1 and CCL17 resulted in a significant decrease in the production of IFN-γ and in the duration of cognate interaction. Taken together, our findings highlight a novel mechanism in which IL-12 enhances chemokine production, prolonging T cell-DC interactions, and increasing the production of IFN-γ by T cells.

It has been shown that IL-12 can influence T cell migration, based on observations that IL-12-conditioned CD8⁺ and CD4⁺ T cells are more responsive to chemokine stimulation (52). Therefore, an alternative explanation to our observed findings was that IL-12 influenced the expression of adhesion molecules or chemokine receptors expressed on CD8⁺ T cells. To address this possibility, we primed T cells by *Listeria*-infected WT, p35⁻/⁻, or p40⁻/⁻ DC for a period of 3 days. The surface expression of CCR5 and CXCR4 (the receptors for the chemokines CCL1 and CCL17, respectively) as well as CCR3 and CXCR5, two chemokines known to be important for the development of T cell responses (52, 54-59), were monitored. We found no difference in the expression of these molecules over the 3-day priming period, regardless of the presence of IL-12. Additionally, expression of CD11a, CD69, and CD44, known mediators of adhesion during T cell activation (3, 60-65), were determined on CD8⁺ T cells on days 1, 2, and 3 of priming by flow cytometry. The expression levels of these molecules were also not different in the presence or absence of IL-12 or IL-23 (data not shown). In addition, we analyzed whether IL-12 increased the surface expression of ICAM-1 on *Listeria*-infected DC. WT, p35⁻/⁻, or p40⁻/⁻ DC were infected with Lm and, on days 1, 2, and 3 after infection, the surface expression of ICAM-1 was measured via flow cytometry. The expression of ICAM-1 was not influenced by IL-12 or IL-23 (data not shown).

It has been determined that long duration interactions between CD8⁺ T cells and DC precedes effective activation of these cells in vivo (21). The majority of these extended interactions occur within the initial 48 h of encounter (21). This observation correlates with our results, which illustrate that the expression of the IL-12 receptor by CD8⁺ T cells peaks within the first 2 days of priming and is down-regulated after this period (data not shown). These observations may explain why CD8⁺ T cells are more sensitive to IL-12 within the first 2 days of activation when T cells are engaged in long duration interaction with DC.

The roles of CCL1 and CCL17 in immune responses are only beginning to be delineated, yet several recent reports indicate that these chemokines play important roles in T cell activation. Following in vivo infection with Lm, Allerink et al. (66) observed an accumulation of CCL17-producing DC in the lymph nodes and nonlymphoid organs of infected mice. In addition, bone marrow-derived DC producing CCL17 were better stimulators of naive CD4⁺ T cell proliferation and IFN-γ production in vitro. Mice deficient in CCL17 production exhibited delayed allograft rejection and reduced hypersensitivity responses in vivo. Based on the localization of CCL17-producing DC and their ability to promote Th1 immune responses by CD4⁺ T cells, these DC are likely to be critical in the induction of anti-listerial CTL responses. Additionally, patients with atopic dermatitis were found to have significantly higher CCL1 levels when compared with the levels found in normal skin and other skin diseases (67). This chemokine, along with CXC12, induced migration of T cells and DC, providing a potential mechanism that would be important in linking innate and adaptive immune responses, perhaps resulting in disease progression (67). Thus, our observation that these chemokines enhance IFN-γ production by CD8⁺ T cells adds to the growing body of literature demonstrating the importance of these chemokines in the development of T cell responses.

Interestingly, while CCL1 and CCL17 were shown to regulate the level of IFN-γ produced by CD8⁺ T cells, we observed little effect of these chemokines on this response in CD4⁺ T cells. This difference may be due to distinct repertoires of chemokine receptors expressed by the two T cell types or to differential requirements for the duration of cognate interaction. These questions are the focus of ongoing study.

Even though IL-12 augmented IFN-γ production through the mechanisms described above, we found that it did not play a significant role in increasing T cell proliferation. Thus, we have concluded that transient interaction between CD8⁺ T cells and Ag-bearing DC are sufficient to induce T cell proliferation, which is in agreement with published literature (21). One potential explanation for why IL-12 does not play a significant role in augmenting T cell proliferation when these lymphocytes are primed only by DC could be due to DC potency as APCs (14, 15, 17, 68-70). *Listeria*-infected DC express high levels of costimulatory molecules such as CD80 and CD86 which augments CD8⁺ T cells proliferation and cytokine production (14, 17). In addition, Lm-infected DC secrete cytokines such as IFN-β that can also be a potent signal to T cells (14, 18-20, 71). Therefore, we feel that the effect of IL-12 on CD8⁺ T cell proliferation changes based on other factors present during priming (the level of costimulation, other cytokines, and chemokines) that could play redundant roles with IL-12 in augmenting this process (72-75).

Because IL-12 shares the p40 subunit with IL-23, we hypothesized that IL-23 could potentially augment the duration of interaction between T cells and DC, correlating with the increased IFN-γ production we observed. In our study, we found that IL-23 produced by DC did not augment IFN-γ production by CD8⁺ T cells or influence the interactions between CD8⁺ T cells and DC during priming. From these observations, we conclude that IL-12 has a greater impact on naive CD8⁺ T cell activation than does IL-23. However, since IL-23 activates multiple immune cells, its in vivo role in the control of infection by intracellular bacterial pathogens and T cell activation warrants further investigation.

The observation that CCL17 is produced by DC in response to Lm infection in vivo was demonstrated several years ago (66). Our in vivo data confirm this finding and extend our understanding of the mechanistic basis of this response by demonstrating that this chemokine is produced by DC in an IL-12-dependent manner in response to listerial infection (Fig. 6). This result suggests that following in vivo infection with *Listeria*, CD8⁺ T cells are likely to remain in long-term cognate interaction with Ag-bearing DC due to IL-12 and CCL1 and CCL17 production, enhancing their expression of IFN-γ and improving the ability of the host to clear the infection.
Our study has demonstrated a previously unrecognized mechanism through which IL-12 regulates CD8+ T cell activation. Our data, along with previously published studies, support the hypothesis that the delivery of IL-12 by DC to CD8+ T cells occurs most efficiently when these immune cells are in close proximity and when a large percentage of CD8+ T cells express the receptor for this cytokine. These observations further support our model in which IL-12 (through the increased production of CCL1 and CCL17) increases the physical interactions between CD8+ T cells and DC when the T cells are most responsive to this cytokine. However, it remains to be determined whether IL-12 and IL-23 produced by DC affect the generation of potent memory or secondary effector CD8+ T cell responses in the context of a bacterial infection. In addition, it will be important to determine whether CCL1 and CCL17 govern naive CD8+ T cell activation and generation of memory CTL in vivo, which could have major implications for vaccine design.

Disclosures

The authors have no financial conflict of interest.

References

ROLE OF CCL1 AND CCL17 IN CD8+ T CELL ACTIVATION


