Absence of CC Chemokine Ligand 2 Results in an Altered Th1/Th2 Cytokine Balance and Failure to Expel Trichuris muris Infection

Matthew L. deSchoolmeester, Matthew C. Little, Barrett J. Rollins and Kathryn J. Else

_J Immunol_ 2003; 170:4693-4700; doi: 10.4049/jimmunol.170.9.4693
http://www.jimmunol.org/content/170/9/4693

References
This article cites 47 articles, 27 of which you can access for free at:
http://www.jimmunol.org/content/170/9/4693.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
Absence of CC Chemokine Ligand 2 Results in an Altered Th1/Th2 Cytokine Balance and Failure to Expel Trichuris muris Infection

Matthew L. deSchoolmeester,* Matthew C. Little,* Barrett J. Rollins,† and Kathryn J. Else*

Despite a growing understanding of the role of cytokines in immunity to intestinal helminth infections, the importance of chemokines has been neglected. As a chemokine with both chemotactic properties and an ability to shape the quality of the adaptive immune response, CC chemokine ligand 2 (CCL2) was investigated as an attractive candidate for controlling resistance to these types of infection, which require highly polarized Th cell responses. We show here for the first time that CCL2 plays an important role in the development of resistance to infection by the gastrointestinal nematode Trichuris muris. Thus, in the absence of CCL2, worm expulsion does not occur, and the lymph node draining the site of infection becomes a Th1-promoting environment. Elevated levels of IL-12 are produced by polarizing APCs, and the composition of the APC environment itself is perturbed, with reduced numbers of macrophages. *The Journal of Immunology, 2003, 170: 4693–4700.

Trichuris muris is a natural mouse model of the gastrointestinal nematode parasite Trichuris trichiura, one of the most prevalent human helminth infections worldwide. Strain variation in the mouse gives a spectrum of phenotypes varying from resistant strains such as BALB/c and C57BL/6, in which the parasite is quickly expelled, to a completely susceptible strain such as AKR, in which suck adult worms survive in the intestine for many weeks or months (1). It is now well established that a Th2-dominated immune response, characterized by the production of IL-4, IL-5, IL-9, and IL-13, is an absolute requirement for worm expulsion (2–5), and the development of a Th1 response associated with high levels of IFN-γ and IL-12 leads to host susceptibility (6, 7). For example, blocking the interaction of IL-4 with the IL-4R using an anti-IL-4R Ab induces susceptibility in normally resistant BALB/K mice, whereas injection of an IL-4 complex into susceptible AKR mice causes worm expulsion (2). BALB/K mice also become susceptible to infection following administration of IL-12 (7). Despite this knowledge, the expulsion mechanism responsible for the elimination of T. muris from its host is not yet understood, although the roles of mast cells, eosinophils, Ab, and CD4+ T cell-mediated cytotoxicity are known not to be essential individually (8–10).

Recently, an increasing number of chemokines have been shown to play important roles in shaping the adaptive immune response in addition to their widely detailed chemotactic properties. The CC chemokine ligand (CCL)2, previously defined as monocyte chemotactic protein-1, was originally described as being chemotactic for monocytes (11–13), but is now known to also be chemotactic for human NK cells (14, 15), memory T cells (16), and basophils (17). In the mouse, CCL2 is a functional monocyte chemotactant in vivo (18–22), and CCL2-deficient mice are unable to recruit monocytes to sites of inflammation in a number of models, including to the peritoneum after thioglycolate administration (23). Importantly with regard to the development of a Th2-biased immune response, CCL2 causes a decrease in IL-12 production from activated macrophages and monocytes (24, 25). Furthermore, the stimulation of naive T cells from OVA-specific TCR transgenic mice in the presence of CCL2 induces IL-4 production and the development of a Th2 phenotype (26, 27), and CCL2 stimulates IL-4 production from Th2-differentiated cells, suggesting a possible positive feedback mechanism for the maintenance or enhancement of Th2 responses (28). Analysis of the CCL2-deficient mouse revealed an inability to mount a Th2 response following OVA sensitization and challenge (29). Interestingly, this was not due to abnormal T cell migration, suggesting a role for CCL2 in adaptive immunity through control of Th cell differentiation as well as in innate immunity through effects on monocyte recruitment.

With regard to models of infection, CCL2 is produced in response to the intestinal nematode, Trichinella spiralis, with expression found in the serum and locally at the site of infection, in the jejunum (30–32). CCL2-deficient mice are resistant to Leishmania major infection, a model that requires a Th1 immune response for resolution (29), and the induction of lung granulomas by Schistosoma mansoni egg Ag results in elevated levels of CCL2 in whole lung tissue (33). A number of reports detail CCL2 being produced in human and mouse intestinal mucosa and associated tissues (34, 35), leading to increased IL-4 and decreased IL-12 production. Furthermore, human lamina propria macrophages, endothelial cells, and intestinal epithelial cells (36) and mouse mesenteric lymph node (MLN) cells and Peyer’s patch cells (35) all have the potential to make CCL2. Therefore, it was decided to further investigate the role of this chemokine in intestinal infection.

It is reported here that mice naturally resistant to T. muris produce higher levels of CCL2 than susceptible mice following infection, and significantly, CCL2-deficient animals are totally unable to expel T. muris infection. This susceptible phenotype is associated with a reduced Th2 response, characterized by low IL-4...
and high IFN-γ and IL-12. Macrophage recruitment to the large intestine and local draining node (MLN) was also decreased in the CCL2-deficient mouse, and relatively fewer CD4+ cells were present in the MLN.

Materials and Methods

Animals

Breeding pairs of SCYA2+/− (CCL2-deficient) (23, 37) were provided by B. Rolls (Dana Farber Cancer Institute, Boston, MA) and bred in-house at University of Manchester. The appropriate control mice, C57BL/6, as well as AKR and BALB/c mice were purchased from Harlan U.K. (Biostate, U.K.). Male mice were used in all experiments. Mice were infected with T. muris when 8–10 wk old.

Parasite

The maintenance of T. muris, the method of infection, and the production of excretory/secretory (E/S) protein were previously described (38). Infections were designed to give each animal ~100–150 infective eggs. Mice were sacrificed at various time points after infection, and the worm burden in the large intestine was assessed as previously described (39).

Culture of MLN cells for in vitro cytokine measurement

MLN cell suspensions from infected or control mice were prepared as described previously (6, 40). Briefly, 5 × 10^6 total MLN cells were resuspended in RPMI 1640 medium supplemented with 5% FCS, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin (all from Invitrogen, Paisley, U.K.), and 60 μM monothioglycerol (Sigma-Aldrich, Poole, U.K.). MLN cells were stimulated with 50 μg/ml T. muris E/S Ag in 48-well plates (Helena Biosciences, Sunderland, U.K.), with supernatants harvested after either 24 or 48 h, as described in the figure legends, and stored at −20°C until use.

Cytokine and chemokine ELISAs

Cytokines were analyzed by sandwich ELISA as previously described (40). The mAb pairs used were: IFN-γ, R4-6A2 and XMG1.2; IL-4, 11B11 and BVD-24G.2; IL-5, TRFK5 and TRFK4 (all from BD Biosciences, Oxford, U.K.); IL-9, 249.2 (E. Schmidt and J. van Snick) and DC9302C12 (BD Biosciences); and IL-12, C15.6 (G. Trinchieri, Schering-Plough, Dardilly, France) and C17.8 (BD Biosciences). All detection Abs were biotinylated and streptavidin-peroxidase (Roche, Sussex, UK.). ABTS (Sigma-Aldrich) was used as a substrate, and plates were read at 405 nm. IL-13 was assayed using the Quantikine M ELISA kit (R&D Systems, Abingdon, U.K.) following the manufacturer’s instructions. Measurements of macrophage inflammatory protein-1α (CCL3) and CCL2 were performed using the DuoSet ELISA development system (R&D Systems) and the OpEIA mouse CCL2 set (BD Biosciences), respectively.

Cincinnati cytokine capture assay

The reagents and protocol for performing the in vivo cytokine capture assay were provided by F. Finkelman (University of Cincinnati, Cincinnati, OH). The method used was previously described (41). Briefly, naive or T. muris-infected mice were injected with biotinylated anti-IL-4 (clone BVD-1D11) 24 h before sacrifice. Serum was collected and diluted 1/2 for measurement of IL-4 by ELISA. ELISA plates were coated with anti-IL-4 capture Ab (clone BVD6-24G.2) overnight and blocked with 10% FCS in PBS. A standard was prepared by mixing IL-4 with biotinylated BVD-1D11 for 3 min before diluting to 100 ng/ml. Samples and standards were then assayed in duplicate using buffers and reagents described previously (40).

Immunohistochemistry

Mice were killed on days 13, 21, and 35 postinfection (p.i.) with T. muris. Age-matched naive controls were killed on day 21. Approximately 8 mm of the large intestine, juxtaposed to the distal cecum, was removed, fixed in 10% formaldehyde in PBS for 20 min at 37°C. Following a further wash in PBS, nonspecific binding sites in the sections were blocked using 20% normal rat serum (Sigma-Aldrich) in PBS for 1 h at room temperature. Endogenous avidin and biotin binding sites were blocked using a commercial kit according to the manufacturer’s instructions (Vector Laboratories, Burlingame, CA). The sections were incubated with either 4 µg/ml biotinylated rat anti-mouse (1D11 for 3 min before diluting to 100 ng/ml. Samples and standards were then read at 405 nm. IL-13 was assayed using the Quantikine M ELISA kit from R&D Systems, Abingdon, U.K.; and used in conjunction with streptavidin-peroxidase (Roche, Sussex, U.K.). BVD-24G.2; IL-5, TRFK5 and TRFK4 (all from BD Biosciences, Oxford, U.K.). IL-9, 249.2 (E. Schmidt and J. van Snick) and DC9302C12 (BD Biosciences); and IL-12, C15.6 (G. Trinchieri, Schering-Plough, Dardilly, France) and C17.8 (BD Biosciences). All detection Abs were biotinylated and streptavidin-peroxidase (Roche, Sussex, UK.). ABTS (Sigma-Aldrich) was used as a substrate, and plates were read at 405 nm. IL-13 was assayed using the Quantikine M ELISA kit (R&D Systems, Abingdon, U.K.) following the manufacturer’s instructions. Measurements of macrophage inflammatory protein-1α (CCL3) and CCL2 were performed using the DuoSet ELISA development system (R&D Systems) and the OpEIA mouse CCL2 set (BD Biosciences), respectively.

Cincinnati cytokine capture assay

The reagents and protocol for performing the in vivo cytokine capture assay were provided by F. Finkelman (University of Cincinnati, Cincinnati, OH). The method used was previously described (41). Briefly, naive or T. muris-infected mice were injected with biotinylated anti-IL-4 (clone BVD-1D11) 24 h before sacrifice. Serum was collected and diluted 1/2 for measurement of IL-4 by ELISA. ELISA plates were coated with anti-IL-4 capture Ab (clone BVD6-24G.2) overnight and blocked with 10% FCS in PBS. A standard was prepared by mixing IL-4 with biotinylated BVD-1D11 for 3 min before diluting to 100 ng/ml. Samples and standards were then assayed in duplicate using buffers and reagents described previously (40).

Immunohistochemistry

Mice were killed on days 13, 21, and 35 postinfection (p.i.) with T. muris. Age-matched naive controls were killed on day 21. Approximately 8 mm of the large intestine, juxtaposed to the distal cecum, was removed, fixed in 10% formaldehyde in PBS for 20 min at 37°C. Following a further wash in PBS, nonspecific
The peak of CCL2 production in AKR mice was later, on day 28 p.i., and was less than that measured in the BALB/c mice on day 21 p.i. (118.1 vs 372.9 pg/ml). In contrast, CCL3 was present at significantly higher levels in E/S-stimulated MLN supernatants from Th1-dominated AKR mice than in those from Th2-dominated BALB/c mice from day 21 p.i. and remained higher through the remainder of the time course (Fig. 2B; \( p < 0.05 \)). Levels of CCL3 increased only slightly in BALB/c mice during the course of infection. The time course of production of CCL2 and CCL3 was identical with that for the cytokines shown in Fig. 1, A and B, i.e., the first measurable increase over background was about day 21 p.i. It appears, therefore, that following infection with T. muris, CCL2 and CCL3 are associated with Th2- and Th1-dominated immune responses respectively.

**CCL2-deficient mice fail to expel T. muris and exhibit diminished Th2 cytokine responses**

To determine the importance of CCL2 in the Th2-driven protective immunity in response to T. muris infection, mice of the normally resistant C57BL/6 strain in which the gene for CCL2 (SCYA2) has been disrupted, were utilized. There was a highly significant difference in the ability of these mice to expel T. muris compared with the C57BL/6 wild type controls. Wild type mice harbored larvae at days 12 and 21 p.i. but had completely cleared the infection by day 35 p.i. (Fig. 3). CCL2-deficient mice, however, maintained a parasite burden on day 35 p.i. with the presence of fecund, adult worms indicating complete susceptibility to infection.

To investigate the Th1/Th2 status of CCL2-deficient mice, MLN cells were recovered from naive and infected animals and stimulated with E/S Ag, and the production of IFN-\( \gamma \), IL-4, IL-5, IL-9, IL-12, and IL-13 was assessed by ELISA. The Th2 cytokines IL-4, IL-5, IL-9, and IL-13 was present at higher levels in C57BL/6 wild-type mice than in CCL2-deficient animals. Although these differences did not reach statistical significance, after in vitro re-stimulation with E/S Ag, strong consistent trends were observed in two separate experiments, with reduced Th2 cytokine levels in CCL2-deficient mice. Also, restimulation of cells with Con A (a more potent method of restimulating cells that have been primed

![FIGURE 1. Worm burden and cytokine responses in BALB/c and AKR mice following infection with T. muris. Mice (three per group) were infected on day 0 with ~100 embryonated eggs and were sacrificed on days 0, 3, 7, 10, 14, 21, 28, and 35 p.i. A, The number of T. muris inhabiting the large intestine was assessed from day 14 p.i. Single-cell suspensions of MLN cells from all time points were cultured for 24 h, and the supernatant was recovered for analysis of cytokines by ELISA. B, IFN-\( \gamma \); C, IL-9. The limits of detection of the assays were: IFN-\( \gamma \), 0.4 ng/ml; and IL-9, 1.6 U/ml. Results are expressed as the mean \( \pm 1 \) SD.](http://www.jimmunol.org/)

![FIGURE 2. CCL2 and CCL3 levels in BALB/c and AKR mice following infection with T. muris. Mice (three per group) were infected on day 0 with ~100 embryonated eggs and were sacrificed on days 0, 3, 7, 10, 14, 21, 28, and 35 p.i. A, Serum collected from clotted whole blood was used to measure CCL2 by ELISA. B, Single-cell suspensions of MLN cells were cultured for 24 h, and the supernatant was recovered for measurement of CCL3 by ELISA. The limits of detection of the assays were: CCL2, 10.4 pg/ml; and CCL3, 189.4 pg/ml. Results are expressed as the mean \( \pm 1 \) SD. * \( p < 0.05 \) compared with AKR mice on the same day p.i.)](http://www.jimmunol.org/)

![FIGURE 3. Worm burden in C57BL/6 and CCL2-deficient mice following infection with T. muris. Mice (four or five per group) were infected on day 0 with ~100 embryonated eggs and were sacrificed on days 12, 21, and 35 p.i. The number of larvae (days 12 and 21 p.i.) or adult worms (day 35 p.i.) in the large intestine was determined. Results are expressed as the mean \( \pm 1 \) SD and are representative of five independent experiments. ND, none detected. * \( p < 0.05 \) compared with C57BL/6 mice.)](http://www.jimmunol.org/)
by parasite Ag in vivo (40)) confirmed these trends, with cells from CCL2-deficient MLN producing significantly lower levels of Th2 cytokines (IL-4: CCL2-deficient, 37.5 ± 22.6 pg/ml; C57BL/6, 307.2 ± 205 pg/ml; IL-5: CCL2-deficient, 10 ± 4.7 U/ml; C57BL/6, 69.1 ± 55.1 U/ml; IL-9: CCL2-deficient, 0.3 ± 0.6 U/ml; C57BL/6, 10.0 ± 10.0 U/ml; p < 0.05 for each cytokine). To confirm the decreased ability of MLN cells from CCL2-deficient mice to produce Th2 cytokines following *T. muris* infection, the sensitive CCCA method (41) was used to measure levels of IL-4 in naive wild-type mice (4.29 pg/ml). No IL-4 was detectable in control animals injected with PBS. Importantly, even postinfection, IL-4 levels in CCL2-deficient mice did not reach naive wild-type levels.

IFN-γ was undetectable in naive mice, but was strongly expressed in both wild-type and CCL2-deficient mice following infection (Fig. 4B). Elevated IFN-γ levels are typical of the resistant, but slow expelling, C57BL/6 mouse strain (3, 8) and different from the faster expelling BALB/c mice in which no increase in IFN-γ production occurs (Fig. 1B). However, differences in production of the Th1-associated cytokine IL-12 were seen, with CCL2-deficient mice producing 686.3 pg/ml compared with 277.4 pg/ml for wild-type MLN cells (Fig. 4C; p < 0.05).

The production of CCL2 and CCL3 in *T. muris*-infected AKR and BALB/c mice was associated with Th2 and Th1 immune responses, respectively (Fig. 2, A and B). As CCL2-deficient C57BL/6 mice showed a Th1-biased response (decreased IL-4, increased IL-12), the levels of these chemokines in MLN cell supernatants of CCL2-deficient and wild-type animals were measured. CCL2 was significantly up-regulated in C57BL/6 mice on day 21 p.i. compared with that in naive mice (Fig. 4D; p < 0.001). Although CCL3 was strongly associated with a Th1 response in susceptible AKR mice, there was no difference in the production of this chemokine between CCL2-deficient and wild-type C57BL/6 MLN cells after restimulation with E/S Ag (Fig. 4E).

**FIGURE 4.** Cytokine and chemokine responses in C57BL/6 and CCL2-deficient mice following infection with *T. muris*. Mice (four or five per group) were infected on day 0 with ~100 embryonated eggs and were sacrificed on day 21 p.i. A. Some animals were injected with biotinylated anti-IL-4 Ab 24 h before sacrifice for the measurement of IL-4 in serum by ELISA. Single-cell suspensions of MLN cells from mice not injected with biotinylated anti-IL-4 were cultured for 48 h, and the supernatant was recovered for the measurement of IFN-γ (B), IL-12 (C), CCL2 (D), and CCL3 (E) by ELISA. The limits of detection of the assays were: IL-4, 4.29 pg/ml; IFN-γ, 4.5 ng/ml; IL-12, 240.5 pg/ml; CCL2, 80.1 pg/ml; and CCL3, 6.6 pg/ml. Results are expressed as the mean ± 1 SD. ND, none detected. *p < 0.05; **p < 0.01; ***p < 0.001.

CCL2-deficient mice differ in the cellular composition of the large intestine and MLN both before and after infection

As CCL2 is a chemoattractant for various cell types, including activated T cells, NK T cells, and basophils, and may be the main chemoattractant for monocyte/macrophages under some conditions, the cellular composition of the large intestine was assessed by histological and immunohistochemical methods, and the composition of the MLN was determined by flow cytometry in naive and *T. muris*-infected WT and CCL2-deficient mice. Infection with *T. muris* caused an expansion of crypt goblet cells and an influx of eosinophils and mast cells to the large intestine. There were no differences in goblet cell hyperplasia, eosinophilia, or mastocytosis between wild-type and CCL2-deficient mice after infection (data not shown). Immunohistochemical staining for the macrophage-specific Ag, F4/80, revealed a significant reduction in the infiltration of macrophages to the large intestine following infection of CCL2-deficient mice. Fig. 5A shows intense F4/80 staining in wild-type mice on day 20 p.i., whereas CCL2-deficient mice exhibit much weaker staining (Fig. 5B). Very few macrophages are present in naive tissue (Fig. 5C). To quantify the differences in staining, sections from four animals on days 0 (naive), 13, 20, and 35 p.i. were stained, and the number of macrophages in 20 cecal crypt units (CCU) of three serial sections for each animal was assessed. There were no differences in the number of macrophages in naive animals or on day 13 p.i. (Fig. 6A). However, there were significantly fewer macrophages in the large intestine of CCL2-deficient animals on day 20 p.i. (p < 0.01). This trend was also present on day 35 p.i., but was not statistically significant due to large variation in macrophage number in the wild-type animals. It is also clear from these data that macrophages do migrate into the large intestine under resting and inflammatory conditions, albeit in reduced numbers, in the absence of CCL2 (Figs. 5B and 6A). Immunohistochemical staining of CD4+ T cells showed that there was no difference in the number of cells infiltrating the large intestine of wild-type or CCL2-deficient mice in naive animals or at any time point following infection with *T. muris* (Fig. 6B).

In the MLN, macrophages (CD11b+/F4/80+ cells) and dendritic cells (CD11c+/MHC II+ cells) made up only a small fraction of the total lymphocyte population (Fig. 7A, R1) when assessed by flow cytometry (0.1–2%; data not shown). However, the application of a separate region (Fig. 7A, R2) revealed that the majority of these APCs resided among the larger, more granular cell population, forming between 1 and 10% of this population. Consequently, quantification of macrophage and dendritic cell numbers was performed on this subpopulation. The proportions of macrophages in
the MLN of wild-type and CCL2-deficient mice both before and during infection are shown in Figs. 7 and 8. Fig. 7, B–D, shows isotype control staining and staining for macrophages isolated from MLN of wild-type and CCL2-deficient mice 20 days after T. muris infection. At this time point there were more macrophages in the MLN of wild-type mice (9.92%) than in CCL2-deficient mice.

FIGURE 6. Quantification of macrophage and CD4+ T cell infiltration of the large intestine following T. muris infection. Mice (four per group) were infected on day 0 with ~150 embryonated eggs and were sacrificed on day 20 p.i. Naive mice were sacrificed 20 days after the start of the experiment. A section of the large intestine was recovered and processed for immunohistochemistry. Sections were stained with F4/80 for identification of macrophages. A, Wild-type mice, day 20 p.i.; B, CCL2-deficient mice, day 20 p.i.; C, wild-type naive mice; D, isotype control (performed on wild-type tissue). Original magnification of the sections, ×200. Arrows indicate the anterior portion of T. muris embedded within gut epithelium.

FIGURE 7. Identification of macrophages in single-cell suspensions of MLN cells from T. muris-infected C57BL/6 and CCL2-deficient mice. Mice (four to six per group) were infected on day 0 with ~150 embryonated eggs and were sacrificed on day 20 p.i. Single-cell suspensions of MLN cells were prepared and stained with anti-mouse CD11b-FITC and F4/80-PE for macrophages or the appropriate control Abs. A, A typical forward vs side scatter plot for MLN cells indicating the main lymphocyte population (R1) and the area richest in macrophages (R2). B, Isotype control staining; C, wild-type MLN cells, day 20 p.i.; D, CCL2-deficient MLN cells, day 20 p.i. The number in the upper right quadrant indicates the percentage of double-positive cells present in area R2 of the scatter plot for that cell population. Plots are representative of two independent experiments.
Interestingly, differences were also found in the total cellular compositions of MLNs from infected and naive mice on day 0 with ~150 embryonated eggs and were sacrificed on days 12, 20, and 34 p.i. Naive mice were sacrificed after the final group of infected mice. Single-cell suspensions of MLNs were prepared and stained with anti-mouse CD11b-FITC and F4/80-PE (A), anti-mouse CD11c-FITC and MHC II-PE (B), anti-mouse CD4-FITC (C), or anti-mouse B220-FITC (D). Results are expressed as the mean relative percentage of total cells staining positively ± 1 SD and are representative of two independent experiments. *, p < 0.05; **, p < 0.01 (compared with C57BL/6 mice).

Table I. Flow cytometry data showing the proportions and CD4^+ T cells, B220^+ B cells, and CD11b-F4/80^+ macrophages in the MLN of naive (day 0 p.i.) and T. muris-infected wild-type and CCL2-deficient mice

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>WT</td>
<td>35.0 ± 1.5</td>
<td>31.2 ± 2.5</td>
<td>12.47 ± 0.64</td>
</tr>
<tr>
<td></td>
<td>KO</td>
<td>26.3 ± 2.1^b</td>
<td>40.2 ± 3.6^b</td>
<td>9.07 ± 1.37^b</td>
</tr>
<tr>
<td>12</td>
<td>WT</td>
<td>34.2 ± 2.8</td>
<td>32.6 ± 4.7</td>
<td>6.72 ± 1.75</td>
</tr>
<tr>
<td></td>
<td>KO</td>
<td>28.5 ± 1.1^b</td>
<td>39.3 ± 4.5</td>
<td>8.02 ± 4.21</td>
</tr>
<tr>
<td>21</td>
<td>WT</td>
<td>30.3 ± 1.0</td>
<td>43.4 ± 0.7</td>
<td>8.39 ± 1.43</td>
</tr>
<tr>
<td></td>
<td>KO</td>
<td>25.8 ± 4.4</td>
<td>47.0 ± 5.6</td>
<td>5.99 ± 1.74^c</td>
</tr>
<tr>
<td>35</td>
<td>WT</td>
<td>32.1 ± 3.2</td>
<td>35.2 ± 3.7</td>
<td>19.69 ± 1.96</td>
</tr>
<tr>
<td></td>
<td>KO</td>
<td>19.7 ± 1.9^b</td>
<td>49.9 ± 3.4^b</td>
<td>14.98 ± 2.62^b</td>
</tr>
</tbody>
</table>

The data represents a repeat of the experiment presented in Fig. 8. There were no significant differences in the percentage of CD11c^-MHCII^- dendritic cells at any point in the experiment. Results are expressed as the mean relative percentage of total cells staining positively ± 1 SD (n = 5 mice/group).

Discussion

That a Th2-dominated immune response, characterized by high levels of IL-4, IL-9, and IL-13, is required for the successful clearance of a T. muris infection in mice is well documented (2–5). However, there has been little investigation into the importance of chemokines in the development of immunity to T. muris and to intestinal nematode parasites in general. It is shown here that the levels of two chemokines, CCL2 and CCL3, differ in AKR and BALB/c strains of mice during the course of T. muris infection. These strains, respectively, exhibit susceptibility (Th1 dominated) and resistance (Th2 dominated) to infection. As such they offer oppositely polarized immune responses, allowing study of the roles of various mediators under such conditions.

The levels of CCL2 and CCL3 were assessed at several time points during the course of infection, and we found increased production of CCL2 associated with resistance in BALB/c mice, while levels remained low in AKR mice. In contrast, a dramatic and sustained increase in CCL3 was seen in susceptible AKR mice from day 21 p.i., whereas CCL3 levels did not increase in BALB/c mice over the time course studied. This suggests that CCL2 is associated with a Th2-dominated response (high IL-4, IL-5, IL-9, and IL-13 and low IFN-γ).

MLN lymphocyte population among the cell types that make up the majority of the node population, namely T cells (CD4^+ or CD8^+ cells) and B cells (B220^+ cells). When expressed as a percentage of the total cells in the MLN, CD4^+ cells comprise 32.3 and 28.4% of cells in the MLN of naive wild-type and CCL2-deficient mice, respectively, a small, but statistically significant, difference (p < 0.05) that is maintained postinfection (Fig. 8C and Table I). Thus, following infection the relative percentage of CD4^+ T cells typically decreases in both wild-type and CCL2-deficient mice p.i. (40) (our unpublished observations), only recovering if worms are expelled (i.e., after day 20 p.i. for wild-type animals). In contrast, the relative percentage of CD8^+ cells did not differ between wild-type and CCL2-deficient mice before or postinfection (data not shown). In both strains the percentage of B cells in the MLN is increased over naive levels on days 20 and 34 p.i., with the peak percentage again corresponding to the presence of worms, occurring on day 20 p.i. in wild-type mice and day 34 p.i. in CCL2-deficient mice (Fig. 8D and Table I). This difference may be due to the persistence of worms in CCL2-deficient mice maintaining B cell proliferation in the MLN and is consistent with data comparing other T. muris-resistant and susceptible mouse strains (our unpublished observations).

![FIGURE 8. The cellular compositions of MLNs from infected and naive C57BL/6 and CCL2-deficient mice. Mice (four to six per group) were infected on day 0 with ~150 embryonated eggs and were sacrificed on days 12, 20, and 34 p.i. Naive mice were sacrificed after the final group of infected mice. Single-cell suspensions of MLNs were prepared and stained with anti-mouse CD11b-FITC and F4/80-PE (A), anti-mouse CD11c-FITC and MHC II-PE (B), anti-mouse CD4-FITC (C), or anti-mouse B220-FITC (D). Results are expressed as the mean relative percentage of total cells staining positively ± 1 SD and are representative of two independent experiments. *, p < 0.05; **, p < 0.01 (compared with C57BL/6 mice).](http://www.jimmunol.org/)

Downloaded from http://www.jimmunol.org/ by guest on April 7, 2017
and CCL3 is associated with a Th1 response (high IFN-γ and low IL-4, IL-5, IL-9, and IL-13) following infection with *T. muris*.

This reciprocal relationship between CCL2 and CCL3 has been previously described with regard to T cell cytokine production. Stimulation of OVA-transgenic T cells in the presence of CCL3 leads to IFN-γ production, whereas the addition of CCL2 induces IL-4 production (26, 27). Also, CCL2 increases and CCL3 inhibits the production of IL-4 from differentiated Th2 lymphocytes (28). The granulomatous lung inflammation model is also well characterized. A type 1 (Th1) granuloma can be induced by mycobacterium purified protein derivative, whereas schistosomal egg Ag induces a type 2 (Th2) granuloma. Under such conditions, CCL2 levels are higher in the local draining lymph nodes of mice with type 2 granulomas, and CCL2 inhibits IL-12 production by activated macrophages isolated from these mice (24). Conversely, CCL3 is produced following the induction of a type 1 granuloma (33). Previous work on the roles of chemokines in parasitic infections has associated CCL2 with two other intestinal parasites that provoke a strong Th2 response within the host: *T. spiralis* infection causes an increase in the level of CCL2 in serum (31), and *Nippostrongylus brasiliensis* infection leads to increased CCL2 production by murine intestinal epithelial cells on days 7 and 14 p.i. (42). These data together with the evidence generated from our work with AKR and BALB/c mice (Figs. 1 and 2) led us to question whether CCL2 was involved in generation of the Th2-associated protective immune response to *T. muris*. As shown in Fig. 3, CCL2-deficient mice on a C57BL/6 background were completely susceptible to infection, whereas wild-type mice expelled the parasite between days 21 and 35 p.i. The susceptibility of CCL2-deficient mice was associated with decreased production of Th2 cytokines (Fig. 4A and data not shown). With regard to Th1 cytokine production, there was no difference in the levels of IFN-γ, but there was increased IL-12 in the CCL2-deficient mice. Despite the susceptible phenotype and enhanced Th1 response generated by CCL2-deficient mice there was no increase in CCL3 above the level seen in wild-type mice. Thus, the relationships between CCL2 and resistance (Th2 response) and between susceptibility and CCL3 (Th1 response) seen in BALB/c vs AKR mice (Fig. 2B) were not apparent. This may be due to the higher levels of IFN-γ found in C57BL/6 compared with BALB/c mice, leading to elevated levels of CCL3 in the wild-type mice.

Leukocyte recruitment was investigated in CCL2-deficient mice infected with *T. muris*, as previous reports have described a deficiency in macrophage recruitment in the absence of CCL2 (23, 37). Using immunohistochemistry a decreased accumulation of macrophages was found in the large intestine following *T. muris* infection. Levels in naive animals were equivalent in wild-type and CCL2-deficient mice, revealing that other chemoattractants are sufficient for the infiltration of resting numbers of macrophages to the large intestine (for example, other members of the monocyte chemoattractant protein family, CCL7, CCL8, and CCL12). The number of macrophages in the large intestine of CCL2-deficient mice increased during the course of infection, indicating that CCL2 is not essential for macrophage infiltration. However, as the number of macrophages infiltrating the large intestine of wild-type mice was significantly higher on day 21 p.i. than in mice deficient in CCL2, there is a subpopulation of F4/80+ cells that do require CCL2 for influx to the gut postinfection. The primary role of the macrophage at the site of infection and inflammation is probably that of phagocytosis of dead or dying cells and foreign material to help resolve the inflammatory response and allow repair of damaged tissue. Therefore, it is possible that the reduced number of macrophages found in the large intestine of CCL2-deficient mice following *T. muris* infection leads to decreased phagocytosis and a more chronic inflammatory response. This in combination with the increased levels of Th1-related cytokines might prolong the survival of the worm in the host. Indeed, two bacterial models of inflammation have shown that a lack of monocyte recruitment to the site of infection is responsible for a more chronic pathology developing, although neither of these studies assessed cytokine status following infection (43, 44). Alternatively, the lack of macrophage recruitment to the gut could be seen as an indication of an inadequate immune response being generated in the CCL2-deficient mice, leading to the failure of the mice to expel the parasite. However, the equivalent production of IFN-γ by MLN cells and the influx of CD4+ T cells to the gut suggest that CCL2-deficient mice mount an immune response of equal magnitude to that of wild-type mice in response to *T. muris* infection.

The cellular composition of the MLN, the lymph node draining the large intestine, was also analyzed during the course of *T. muris* infection. At this site there were significantly fewer macrophages, but normal numbers of dendritic cells in the absence of CCL2 in naive mice and on days 20 and 34 p.i. This may therefore result in quantitative or qualitative differences in T and B cell activation in the MLN, producing an immune response biased toward Th1. Indeed, a macrophage trafficking defect in CCR2 knockout mice (the receptor for CCL2) has been shown to be responsible for a reduction in Ag-specific IFN-γ production in lymph nodes (45). Although this study results in mice exhibiting Th2-dominated, rather than Th1-dominated, immune responses, it does set a precedent for the importance of the macrophage in stimulating Ag-specific T cells in the draining lymph node. Interestingly, CCL2 decreases IL-12 production from both monocytes (25) and inflammatory macrophages (24), and also increases IL-4 and decreases IL-12 production by mucosal tissue (35). Consequently, in the absence of CCL2, fewer macrophages are present in the draining lymph node and the site of infection, and the macrophages that are present lack a control mechanism for IL-12 production. They may therefore express increased amounts of this Th1-inducing cytokine, and indeed, our data reveal significantly elevated IL-12 levels in the MLN in the absence of CCL2. In our model system, therefore, the absence of CCL2 may help promote a Th1 response through elevated IL-12 production from APCs, such as the macrophage or dendritic cell.

Fewer CD4+ T cells were found in the MLN, but not the large intestine, of both naive and infected CCL2-deficient mice. This suggests that there is a population of T cells directly or indirectly dependent on the presence of CCL2 for their migration into and/or maintenance in the MLN. CCL2 has been shown to be a chemoattractant for T cells in vitro (16), and several studies have shown a role in vivo. Firstly, overexpression of human CCL2 in mouse type II alveolar epithelial cells leads to an increase in CD3+ T cell accumulation in the lung (21). Secondly, CCL2-deficient mice have decreased lymphocyte infiltration in a model of inflammation (46). Thirdly, treatment with a CCL2-neutralizing Ab decreases T cell recruitment to the lung (47). Although Gu et al. (29) found no difference in the migration of naive T cells to secondary lymphoid organs, our study allows for differences in chemokine receptor expression on naive, activated, and memory T cells also affecting T cell trafficking. Also, three independent in vitro experiments were performed in which MLN cells isolated from *T. muris*-infected wild-type and CCL2-deficient mice were stimulated with E/S Ag in the presence or the absence of CCL2. There were no differences in the production of IL-4, IFN-γ, or IL-12 by MLN cells in vitro (data not shown), suggesting that the effect of CCL2 on cytokine production in MLN is indirect. Thus, in our model system the altered MLN environment of CCL2-deficient mice, containing proportionally fewer CD4+ T cells and macrophages, may underlie
CCL-DEFICIENT MICE ARE SUSCEPTIBLE TO T. muris INFECTION

the failure of CCL2-deficient mice to fully develop a Th2-dominated immune response, resulting in long term persistence. 

Here we show for the first time a requirement for the chemokine CCL2 in protective immunity to an intestinal helminth. CCL2 deficiency is linked to a decreased recruitment of macrophages to both the draining lymph node and the large intestine (the site of infection). In addition, it is shown that the absence of CCL2 leads to significantly elevated levels of IL-12, reduced Th2 cytokine levels, and failure to expel T. muris.

Acknowledgments

We gratefully acknowledge the help of Louise Bell with the collection and analysis of data. We are also indebted to Fred Finkelman for kindly providing the reagents for the Cincinnati cytokine capture assay, and all the staff at the Biomedical Services Unit at University of Manchester.

References