Disruption of CD40-CD40 ligand pathway inhibits the development of intestinal muscle hypercontractility and protective immunity in nematode infection


Disruption of CD40-CD40 ligand pathway inhibits the development of intestinal muscle hypercontractility and protective immunity in nematode infection. Am J Physiol Gastrointest Liver Physiol 288: G15–G22, 2005. First published August 12, 2004; doi:10.1152/ajpgi.00159.2004.—In our previous studies, we demonstrated that during *Trichinella spiralis* infection, T helper (Th) 2 cells contribute to the development of intestinal muscle hypercontractility and worm expulsion from the gut via STAT6. In addition, we have linked the altered muscle contractility to the eviction of the parasite and thereby to the host defense. However, the initial events linking infection to the development of muscle hypercontractility are poorly understood. In this study, we examined the contribution of CD40-CD40 ligand interaction in the development of intestinal muscle hypercontractility, in monocyte chemoattractant protein-1 (MCP-1) production, and in the Th2 response in CD40 ligand-deficient (CD40L−/−) mice infected with *T. spiralis*. Expulsion of intestinal worms was substantially delayed in CD40L−/− mice compared with the wild-type mice after *T. spiralis* infection. Consistent with delayed worm expulsion, there was a significant attenuation of intestinal muscle contractility in CD40L−/− mice. Infected CD40L−/− mice also exhibited marked impairment in the production of MCP-1, IL-4, IL-13, IgG1, IgE, and mouse mucosal MCP 1 (MMCP-1), and in goblet cell response. These results demonstrate that CD40-CD40 ligand interaction plays an important role in MCP-1 production, Th2 response, intestinal muscle hypercontractility, and worm expulsion in nematode infection. The present data suggest that the early events leading to the generation of Th2 response include CD40-CD40 ligand interaction, which subsequently influences the production of Th2 cytokines, most likely via upregulation of MCP-1.

CD40 ligand; monocyte chemoattractant protein-1; T helper 2 cells; muscle; *Trichinella spiralis*; host defense

T Helper (Th) 2 cells play an essential role in the development of host protective immunity in many intestinal nematode infections including *Trichinella spiralis* (12, 14, 17). STAT6 is critical in the differentiation of Th cells toward the Th2 phenotype (43), and in a previous study (28) using STAT6-deficient (STAT6−/−) mice, we showed a significant attenuation in infection-induced intestinal muscle contractility in STAT6−/− mice compared with the wild-type mice, which was accompanied by delayed worm expulsion from the intestine. We were also able to demonstrate the presence of IL-4 and IL-13 in the muscularis externa layer during the primary infection with *T. spiralis*. These observations clearly indicated that the Th2 cytokines IL-4 and IL-13, acting via STAT6, mediate the development of nematode infection-induced intestinal muscle hypercontractility, which in turn contributes to the host defense by effective expulsion of worms. Nevertheless, the initial events linking infection to the development of the changes in intestinal muscle function are poorly understood. Moreover, in contrast to an increasing understanding of the mechanisms by which bacteria, virus, or protozoa generates Th1 type responses, little is known about the initiation of Th2 type responses activated by nematode infection, and this remains an exciting area to explore.

The differentiation of naive T cells into effector cells requires antigen recognition and signals provided by costimulatory molecules expressed on antigen-presenting cells (APCs). Among the costimulatory pathways, the CD40-CD40 ligand (CD40L) pathway plays an important role in T cell responses (20). Although the mechanisms by which this pathway influences T cell responses are not clear, upregulation of other costimulatory pathways or enhancement of chemokines and cytokines production are likely relevant. CD40 is a cell surface receptor that belongs to the TNF-R family; is constitutively expressed on APCs, whereas CD40L is a member of TNF family; and is induced on T cell activation (47). Interaction between CD40 and CD40L has been shown to regulate both humoral and cellular immune responses (20, 47). Demonstration of the critical role of CD40-CD40L interaction in T cell-dependent B cell responses was revealed by the findings that the hyper-IgM syndrome, an X-linked immunodeficiency disorder, is due to the mutation of the CD40L gene. This disease is characterized by a severe impairment in T cell-dependent antibody responses with no B cell memory, defective germinal center formation, and a markedly reduced antibody repertoire (4, 15). Mice deficient in CD40 and in CD40L genes demonstrate a phenotype comparable to that of the individuals suffering from the hyper-IgM syndrome (25, 40, 49). A contribution of CD40-CD40L interactions to the process of T cell priming, differentiation, and effector functions has been also described (19, 36).

Manipulation of CD40-CD40L pathway in animal models by the use of mice deficient in CD40 or CD40L, as well as the use of neutralizing antibodies, enables study of the role of...
CD40-CD40L interaction in several disease models, including transplantation, autoimmunity, and infectious diseases (47). Most of the studies on the CD40-CD40L interactions during infection have focused on the infective agents that generate Th1 responses (18) and have convincingly demonstrated the importance of this interaction in the development of Th1 immune responses during infection. In contrast, there are few studies that have examined CD40-CD40L interactions in the development of Th2-based immune responses and host defense. Recently, a role for CD40-CD40L interaction in the development of Th2-mediated host protective immunity has been described in mice infected with Schistosoma mansoni (35).

Monocyte chemoattractant protein-1 (MCP-1) is a member of the C-C chemokine family and acts on T cells, monocytes, and natural killer cells. Studies in MCP-1-deficient mice suggest that this chemokine is involved in the development of Th2 type immune response and in intestinal worm expulsion during nematode infection (11).

In the present study, we examined the contribution of CD40-CD40L interaction in the development of intestinal muscle hypercontractility, in MCP-1 production, and in the Th2 response in T. spiralis infection using CD40L-deficient (CD40L−/−) mice. We reasoned that if CD40-CD40L interaction and MCP-1 are required for the development of Th2 response, then interruption of CD40-CD40L interaction should not only prolong the infection but should also attenuate infection-induced muscle hypercontractility and inhibit MCP-1 production. For the first time, we demonstrate that interaction between CD40 and CD40L is required for the generation of infection-induced intestinal muscle hypercontractility during the primary infection with T. spiralis. Our results also confirm the importance of CD40-CD40L interaction in the development of Th2 response and host defense. The results also suggest an important role of CD40-CD40L interaction in the production of MCP-1, which subsequently may influence the generation of Th2 response during nematode infection.

MATERIALS AND METHODS

Animals. CD40L−/− mice on C57BL/6 background and C57BL/6 (CD40L+/+) were purchased from the Jackson Laboratories (Bar Harbor, ME). Mice were kept in sterilized, filter-topped cages and fed autoclaved food in the animal facilities of McMaster University. Only the male mice were used at the age of 8–10 wk. The protocols employed were in direct accordance with guidelines drafted by the McMaster University Animal Care Committee and the Canadian Council on the Use of Laboratory Animals.

Parasitological techniques. T. spiralis parasites used in the study originated in the Department of Zoology at the University of Toronto, and the colony was maintained through serial infections alternating between male Sprague-Dawley rats and male CD1 mice. The larvae were obtained from infected rodents 60–90 days postinfection, using a modification (48) of the technique described by Castro and Fairbairn (6). Mice were killed at various time points after infection. Adult worms were recovered from mice after the intestine had been opened longitudinally, rinsed, and placed in Hank’s balanced salt solution for 3 h at 37°C. Worms were counted under a dissecting microscope.

Measurement of muscle contraction in intestinal muscle strips. Preparation of intestinal muscle strips for muscle contractility experiments and analysis of carbachol-induced contraction have been described previously (45). Briefly, the jejunum was removed and placed in oxygenated (95% O2-5% CO2) Krebs solution, and 1-cm sections of whole gut were cut from the jejunum. The lumen of each segment was flushed with Krebs buffer before the insertion of short (2–3 mm) lengths of Silastic tubing (0.065 in. OD, 0.030 in. ID; Dow Corning, Midland, MI) into the open ends of the gut segments. Tubing was then tied in place with surgical silk. The insertion of the tubing was found to maintain patency of the gut segments during experiments. Segments were then hung in the longitudinal axis and attached at one end to a force transducer (model FT03C; Grass, Quincy, MA), and responses were recorded on a Grass 7D polygraph. Tissues were equilibrated for 30 min at 37°C in Krebs buffer and oxygenated with 95% O2-5% CO2 before starting the experiment. The previously identified optimal tension was then applied in carbachol dose-response experiments before the addition of the first dose of carbachol (45). Previous experiments indicated that this was optimal tension to determine the maximal responsiveness of both control and inflamed tissues. After the application of tension, gut segments were exposed to different concentrations of carbachol. After the maximal response to each dose was obtained, tissues were rinsed twice and equilibrated in fresh Krebs solution for 15 min before addition of the next dose. Contractile responses to carbachol were expressed as milligrams of tension per cross-sectional area as described previously (45). For each mouse, the mean tension was calculated from at least three segments.

Mouse MCP-1 ELISA. Levels of mouse MCP-1 (MCP-1) in serum were measured with an MCP-1 ELISA kit purchased from Moredun Animal Health (Penicuik, UK) as previously described (23).

Evaluation of in vitro IL-4, IL-13, MCP-1, and IFN-γ production from spleen cells. Single-cell suspensions of spleen were prepared in RPMI 1640 containing 10% FCS, 5 mM l-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, 25 mM HEPES, and 0.05 mM 2-mercaptoethanol (all GIBCO-BRL). Cells (1 × 10⁷) were incubated in the presence of 5 µg/ml concavalin A (Con A) or 50 µg/ml T. spiralis antigen.

Culture supernatants were harvested after 24 h and were stored at −20°C. IL-4, IL-13, MCP-1, and IFN-γ levels in the supernatants were measured by enzyme immunoassay technique using commercially available kits purchased from R&D Systems (Minneapolis, MN).

Investigation of intestinal tissue IL-4 level. Frozen intestinal tissues were homogenized in lysis buffer containing protease inhibitor cocktail (Sigma). The homogenates were freeze-thawed three times and centrifuged, and then supernatant was collected and stored at −20°C until analyzed.

IL-4 level in the supernatant was measured by enzyme immunoassay technique using a commercially available kit purchased from R&D Systems. Concentration of protein in the intestinal tissue was determined by a commercially available DC Protein Assay kit (Bio-Rad), and the amount of IL-4 in the tissues was expressed per milligram of tissue protein.

Determination of serum IgG1, IgG2a, and IgE levels. Levels of parasite-specific IgG1, IgG2a, and total IgE responses were determined as previously described (16) with slight modification. Briefly, wells of a 96-well microtiter plate were coated with 5 µg T. spiralis antigen in 0.05 M carbonate buffer (pH 9.6) and incubated overnight at 4°C. After being washed with saline containing 0.1% Tween-20, free binding sites of each well were blocked with blocking buffer (100 ml of washing buffer and 2 g of BSA). After being washed, serum samples diluted with blocking buffer were applied. After incubation at 37°C for 1 h, the wells were washed, and then peroxidase-conjugated anti-mouse IgG1 or IgG2a were added to the wells. The plate was incubated at 37°C for 1 h. After wells were washed, a mixture of H₂O₂ and substrate, 2,2-azino-di-(3-methylbenzthiazoline sulphonic acid) (ABTS; Sigma, St. Louis, MO) in substrate buffer was added to each well, incubated for 30 min, and then the absorbance was read at the wavelength of 405 nm.

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For determination of total IgE, wells were coated with monoclonal anti-mouse IgE antibody (5 μg/ml) and incubated overnight at 4°C. After being washed and blocked, serum samples and IgE standard, diluted in the blocking agent, were added to the wells. After incubation, wells were washed and peroxidase-conjugated anti-mouse IgE was applied to the wells. After wells were washed, a mixture of H₂O₂ and substrate ABTS was added to each well and incubated for 30 min; the absorbance was then read at the wavelength of 405 nm.

Histology. A segment (1 cm in length) of small intestine was fixed in 10% neutral buffered formalin (NBF) and processed by using standard histological techniques. Sections from NBF were stained with periodic acid Schiff reaction for counting intestinal goblet cells. The numbers of goblet cells were expressed per 10 villus crypt units.

Statistical analysis. Data were analyzed by using Student’s t-test, with P < 0.05 considered significant. All results are expressed as means ± SE.

RESULTS

Expulsion of worms is inhibited in CD40L −/− mice infected with T. spiralis. To elucidate the role of CD40-CD40L interaction in T. spiralis expulsion, CD40L +/+ and CD40L −/− mice were infected with T. spiralis larvae and killed on different days after infection. Almost all worms were expelled from CD40L +/+ mice by day 21 postinfection. However, worm expulsion was significantly delayed in CD40L −/− mice. We recovered a significantly higher number of worms from CD40L −/− mice compared with that from CD40L +/+ mice on day 21 postinfection (Fig. 1), indicating prolonged infection in CD40L −/− mice.

CD40-CD40L interaction is required in the development of infection-induced intestinal muscle hypercontractility during T. spiralis infection. The contribution of CD40-CD40L interaction to intestinal muscle contraction during T. spiralis infection was investigated by using CD40L −/− mice. There was no significant difference in intestinal muscle contractility between noninfected CD40L +/+ and CD40L −/− mice. Infec-

![Graph](https://via.placeholder.com/150)

**Fig. 1.** Worm recovery from CD40 ligand (CD40L) +/+ and CD40L deficient (CD40L −/−) mice after *Trichinella spiralis* infection. Mice were infected orally with 375 *T. spiralis* larvae and killed on the days indicated to investigate the worm recovery from intestine. Each bar represents the mean ± SE from 5 animals. *Significantly different between CD40L +/+ and CD40L −/− mice.

![Graph](https://via.placeholder.com/150)

**Fig. 2.** Contraction of intestinal muscle from CD40L +/+ and CD40L −/− mice. Maximum tension generated by intestinal muscle taken from noninfected and *T. spiralis* infected CD40L +/+ and CD40L −/− mice in response to carbachol. CD40L +/+ and CD40L −/− mice were infected orally with 375 *T. spiralis* larvae and killed on the time points indicated. *Significantly different between CD40L +/+ and CD40L −/− mice.

![Graph](https://via.placeholder.com/150)

**Fig. 3.** Absence of CD40-CD40L interaction reduced Th2 type cytokine production during T. spiralis infection. Measurement of in vitro cytokine production from spleen cells by stimulation with Con A and *T. spiralis* antigen, which stimulates T lymphocytes nonspecifically and specifically, respectively, revealed severe impairment of IL-4 and IL-13 production in CD40L −/− mice compared with CD40L +/+ mice after *T. spiralis* infection. We observed high amounts of both IL-4 and IL-13 in CD40L +/+ infected mice. However, production of IL-4 and IL-13 was severely impaired in CD40L −/− infected mice. We observed markedly lower amounts of IFN-γ in infected CD40L +/+ mice compared with IL-4 and IL-13 production, and IFN-γ was further reduced in infected CD40L −/− mice.
To evaluate local Th2 cytokine production, we also measured intestinal tissue IL-4 levels. As shown in Fig. 4, there were significantly lower amounts of intestinal tissue IL-4 in infected CD40L −/− mice compared with that in infected CD40L +/+ mice both on days 7 and 14 postinfection.

**Production MCP-1 impaired in the absence of CD40-CD40L interaction.** Investigation of MCP-1 production in the supernatant of in vitro Con A-stimulated spleen cells demonstrated a significantly reduced level of MCP-1 in CD40L −/− mice compared with CD40L +/+ mice after T. spiralis infection on days 7 and 14 postinfection (Fig. 5), indicating an important role for CD40-CD40L interaction in the production of this chemokine after the infection.

**Absence of CD40-CD40L interaction reduced IgG1 and IgE responses during T. spiralis infection.** We then investigated IgG1, IgG2a, and IgE responses in the serum of CD40L +/+ and −/− mice. We observed significantly higher T. spiralis antigen-specific IgG1 response in CD40L +/+ mice after infection compared with that in CD40L −/− mice. Indeed, the level of IgG1 in CD40L −/− infected mice was almost similar to that in noninfected CD40L +/+ and noninfected CD40 −/− mice (Fig. 6A). There was no significant increase in antigen-specific IgG2a response in both CD40L +/+ and CD40L −/− mice after infection.

Total IgE level in the serum was not detected on day 7 postinfection in either CD40L +/+ and CD40L −/− mice. We observed significantly elevated IgE level on days 14 and 21 postinfection in CD40L +/+ mice compared with that in control noninfected mice. However, CD40L −/− mice failed to exhibit IgE until day 21 postinfection (Fig. 6B).

**Absence of CD40-CD40L interaction attenuated MMCP-1 production and development of intestinal goblet cell hyperplasia during T. spiralis infection.** We then investigated serum MMCP-1 and intestinal goblet cell hyperplasia, which are considered to be Th2-mediated characteristic components of intestinal nematode infection. Serum MMCP-1 levels were undetectable in noninfected mice. Infection with T. spiralis caused a significant increase in serum MMCP-1 concentration in CD40L +/+ mice compared with that in noninfected control. However, the serum MMCP-1 level was significantly lower in CD40L −/− mice after infection on both days 7 and 14 postinfection (Fig. 7).

Markedly increased numbers of goblet cells were observed in the intestine of CD40L +/+ mice on days 7 and 14 postinfection after T. spiralis infection, compared with that in noninfected control. However, we observed a significantly lower number of goblet cells in CD40L −/− mice compared with that in CD40L +/+ mice on day 14 after the infection (Fig. 8).

### Table 1. Cytokine produced by in vitro stimulated spleen cells from CD40L +/+ and CD40L −/− mice infected and noninfected mice.

<table>
<thead>
<tr>
<th></th>
<th>CD40L +/+</th>
<th>CD40L −/−</th>
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<tbody>
<tr>
<td></td>
<td>ConA</td>
<td>Antigen</td>
</tr>
<tr>
<td>IL-4</td>
<td>20.38±3.19</td>
<td>10.02±4.07</td>
</tr>
<tr>
<td>IL-13</td>
<td>105.40±25.31</td>
<td>35.32±8.82</td>
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<tr>
<td>IFN-γ</td>
<td>20.20±3.73</td>
<td>7.99±2.62</td>
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<tr>
<td>Day 7 postinfection</td>
<td></td>
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<tr>
<td>IL-4</td>
<td>611.13±73.16</td>
<td>317.83±37.13</td>
</tr>
<tr>
<td>IL-13</td>
<td>1,230.95±179.60</td>
<td>803.62±97.84</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>64.88±14.83</td>
<td>10.21±1.60</td>
</tr>
<tr>
<td>Day 14 postinfection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-4</td>
<td>206.09±24.22</td>
<td>110.27±10.83</td>
</tr>
<tr>
<td>IL-13</td>
<td>1,066.15±99.48</td>
<td>817.25±59.29</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>19.86±3.76</td>
<td>0±0</td>
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Each value in means ± SE (pg/ml) represents mean ± SE from 5 mice. Spleen cells from noninfected and infected CD40L +/+ and CD40L −/− mice were stimulated with concavalin A (Con A) or Trichinella spiralis antigen for 24 h, and the levels of IL-4, IL-13 and IFN-γ present in the supernatant were investigated by ELISA. *Significantly different between Con A-stimulated spleen cells from CD40L +/+ and spleen cells from CD40L −/− mice. **Significantly different between antigen-stimulated spleen cells from CD40L +/+ and spleen cells from CD40L −/− mice. ***CD40L ligand deficient.
DISCUSSION

The present study demonstrates a critical role for CD40-CD40L interaction in the development of intestinal muscle hypercontractility and in the generation of effective Th2 immune response during infection of mice with *T. spiralis*. We have shown previously that intestinal muscle hypercontractility is mediated largely via STAT6 and Th2 cytokines (28), we interpret the attenuation of muscle contractility as a consequence of an impaired Th2 response due to the absence of CD40-CD40L interaction. It seems likely that CD40-CD40L interaction after infection activates Th cells, and subsequently Th cells differentiate toward Th2 cells via the STAT6 pathway. Disruption of the CD40-CD40L pathway inhibited this process. Results of the present study also show marked attenuation of MCP-1 production in the absence of CD40-CD40L interaction; the absence of MCP-1 may be a reason for the attenuated Th2 response in infected CD40L−/− mice. Taken together, these data suggest that the initial events regulating differentiation of Th cells toward the Th2 pathway in nematode infection include CD40-CD40L interaction and the generation of MCP-1.

Altered intestinal muscle function has been described in rodents after infection by several gastrointestinal nematodes including *Nippostrongylus brasiliensis* (13), *Heligmosomoides polygyrus* (50), *Trichuris muris* (27), and *T. spiralis* (28, 45, 48). We consider this increase in muscle contractility as a critical component of host protection during *T. spiralis* infection. Because the expelled worms in *T. spiralis* infection retain their viability and fecundity, the worms are actually evicted from the gut and this draws attention to the role of the motor system in this process. Previous studies (21, 37) from our laboratory showed regional differences in the intestine in response to inflammation, with muscle from the jejunum exhibiting increased contractility, whereas that from the ileum and colon exhibited reduced contractility. We assumed that the distribution of changes in contractility would create an aboral gradient during infection, thereby enhancing aboral propulsion of luminal contents. If such forces contribute to the host defense against parasites, then one might expect to see a relationship between the magnitude of intestinal muscle hypercontractility and the ability of the host to expel the parasites from the gut. This is indeed the case, with a strong responder to *T. spiralis* infection, such as NIH Swiss mice, which rapidly expel the parasite exhibiting the greater degree of muscle contractility and slow responders, such as B10.BR mice, which expel the parasite slowly, exhibiting a lesser degree of hypercontractility (45).

There is infiltration of intestinal muscle layers by T lymphocytes during *T. spiralis* infection, and ultrastructural analysis revealed close communication between lymphocytes and muscle cells, indicating a direct interaction between these cells (8). Recent work (28) has revealed the presence of IL-4 and IL-13 in the intestinal muscle layer and attenuation of muscle hypercontractility in mice deficient in STAT6, suggesting an important role for Th2 cytokines. It has also been shown that IL-4 and IL-13 enhanced carbachol induces contraction in dispersed...
smooth muscle cells isolated from the jejunum of mice (1). Very recently, it has also been reported that IL-4 and IL-13 play an important role in the generation of intestinal muscle contractility in mice infected with N. brasiliensis and H. polygyrus (50), further supporting the hypothesis that Th2 response mediates intestinal muscle contractility in nematode infection. Previously, we (26) have shown that transfer and overexpression of IL-12 gene during Th2-based T. spiralis infection shifts the immune response toward Th1 and inhibits worm expulsion. In addition, this shift in immune response attenuated infection-induced muscle hypercontractility. Although considering the influence of enteric nerves, interstitial cells of Cajal, and endocrine factors, changes in contractility observed in vitro cannot be extrapolated directly into the in vivo situation. Muscle contractility is an acceptable and convenient method of assessing how inflammatory and immune cells alter this component of intestinal motility. Taken together, these results clearly indicate that the Th2 type immune re-

Fig. 6. Levels of serum immunoglobulins in CD40L +/+ and CD40L −/− mice after T. spiralis infection. Mice were infected orally with T. spiralis and killed 1) on day 14 postinfection to assess serum IgG1 and IgG2a responses and 2) on days 7, 14, and 21 to investigate IgE. Each value represents the mean ± SE from 4 animals.

Fig. 7. Serum mouse MCP-1 (MMCP-1) response in CD40L +/+ and CD40L −/− mice after T. spiralis infection. Mice were infected orally with T. spiralis and were killed on the time points indicated to investigate serum MMCP-1. Each value represents the mean ± SE from 4 animals.

Fig. 8. Intestinal goblet cell response in CD40L +/+ and CD40L −/− mice after T. spiralis infection. Mice were infected with T. spiralis and were killed on days 7 and 14 postinfection to investigate periodic acid Schiff-stained intestinal goblet cell number. Each bar represents the mean ± SE from 5 animals. *Significantly different between CD40L +/+ and CD40L −/− mice.
response is critical for the development of intestinal muscle hypercontractility in this infection and also that the muscle hypercontractility may be an important component in parasite evasion from the intestine.

Th2 cells are not precommitted phenotypes but rather, denote the outcome of a multistep differentiation process by which a common precursor population acquires a distinct cytokine secretion profile. The observation that the deficiency in CD40L in mice is associated with attenuated muscle contractility, and prolonged worm expulsion suggests that the CD40-CD40L interaction is an important event in the early stages of effective protective immunity development after this nematode infection. This requirement is evident in the markedly impaired ability of CD40L−/− mice to produce the Th2 cytokines IL-4 and IL-13 from both in vitro stimulated spleen cells and intestinal tissues, and in their reduced ability to produce antibodies IgG1 and IgE, which are characteristic of Th2-based infection. This observation clearly emphasized the importance of CD40-CD40L interaction in the development of a Th2-type immune response. Recently, an important role of CD40-CD40L interaction in the development of Th2 immune response has been also shown in another helminth infection, Taenia crassiceps (41). The reduction in Th2 cytokine production in the absence of CD40-CD40L interaction was not associated with a shift toward Th1 response, because there was no upregulation in IFN-γ production in CD40L−/− mice. Indeed, we observed a reduction in IFN-γ production in infected CD40L−/− mice, suggesting a difference in activation of the Th2 response between infected CD40L+/+ mice and CD40L−/− mice, rather than a difference due to a shifting of immune response.

Chemokines play important roles in directing cell movements necessary for the initiation of T cell immune responses. They are required to attract APCs to the sites of inflammation, direct APCs to lymphatic vessels, and bring APCs and lymphocytes together within the lymphoid organ (9). Chemokines are also considered important in recruiting appropriate effector cells to the sites of inflammation, including the differential recruitment of Th1 and Th2 cells (42). Recent studies (34) indicate that they also play substantial roles in the regulation of T cell differentiation. We observed a marked reduction in MCP-1 production in the absence of CD40-CD40L interaction in infected CD40L−/− mice after T. spiralis infection. MCP-1 is produced by a variety of cells including dendritic cells, macrophages, endothelial cells, and fibroblasts, and expression is upregulated after exposure to inflammatory stimuli, such as IL-1, TNF-α, and IL-4 (46). Expression of MCP-1 is upregulated in serum and intestinal muscle layer after T. spiralis infection (10, 39). MCP-1 was originally identified as a monocyte-specific chemoattractant but later was shown to act on T cells, mast cells, basophils, and natural killer cells (2, 3, 5, 31). In relation to the polarization of Th cells, MCP-1 has been reported to cause a decrease in IL-12 production from activated macrophages (7), and an increase in IL-4 production from CD4+ T cells (24, 33), suggesting a role of this chemokine in the regulation of Th2 response. MCP-1-deficient mice are resistant to Leishmania major infection, the model that requires Th1 immune response for clearing the infection (22). Recently, it has been reported that in the absence of MCP-1, worm expulsion is inhibited during infection with intestinal nematode T. muris and that this was associated with a shift of immune response toward Th1 type from Th2 type (11). We also observed an enhancement of Th2 response in T. spiralis mice after transfer and overexpression of the MCP-1 gene (29). Thus the reduced level of Th2 response after T. spiralis infection in CD40L−/− mice we observed in this study may be associated with the impairment in MCP-1 production resulting from the absence of CD40-CD40L interaction.

Characteristic components of the Th2-based immune response to nematode infections include mucosal mastocytosis and goblet cell hyperplasia (12, 17). Here, we also investigated the role of CD40-CD40L interaction in the development of mast and goblet cells. Mast cells are generally considered important in host protective immunity in T. spiralis infection, although some controversy exists (30, 44). To investigate the mast cell response, we have quantified the serum levels of MMCP-1, a protein known to be produced by mucus mast cells in vivo (38). Although the level of MMCP-1 was significantly elevated during T. spiralis infection in CD40L+/+ mice, the infection-induced MMCP-1 response was significantly affected in CD40L−/− mice. Similarly, we also observed an inhibition in the development of hyperplasia of mucin-secreting intestinal goblet cells. These observations further support the notion that CD40-CD40L interaction is a major component in the development of effective Th2-mediated host responses. Related to this, it has been recently reported that treatment of mice with anti-CD40L antibody blocked the H. polygyrus-induced elevation of IgG1 and inhibited the development of mucosal mastocytosis and blood eosinophilia (32), further suggesting that CD40-CD40L interaction may be required for the proliferation/differentiation of cells other than B cells.

Taken together, our data provide substantial evidence that the initial events in the generation of Th2-mediated muscle hypercontractility and host protective immunity during nematode infection include the interaction between CD40 and CD40L, which subsequently influence the production of Th2 cytokines, most likely via upregulation of MCP-1. These results are also consistent with our hypothesis that intestinal physiological changes, such as muscle function and the worm expulsion in nematode infection, share a common immunological basis and may be causally linked.

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