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Cysteine Protease B of *Leishmania mexicana* Inhibits Host Th1 Responses and Protective Immunity

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C3H mice infected with *Leishmania mexicana* fail to develop a protective Th1 response, and are unable to cure. In this study, we show that *L. mexicana* cysteine proteases suppress the antileishmanial immune response. Previous studies demonstrated that deletion of the entire multicopy cysteine protease B (CPB) gene array in *L. mexicana* is associated with decreased parasite virulence, potentially attributable to factors related to parasite fitness rather than to direct effects on the host immune response. We now show that C3H mice infected with the *L. mexicana* deletion mutant (Δcpb) initially develop lesions that grow at rates comparable to those of wild-type *L. mexicana*-infected mice. However, in contrast to controls, Δcpb-induced lesions heal with an accompanying Th1 immune response. Lesion resolution was Th1 dependent, as Δcpb-infected IL-12p40−/− and STAT4−/− mice developed high parasite burdens and progressive disease. Moreover, when *L. major* was transfected with a cosmid expressing multiple *L. mexicana* CPB genes, this parasite induced a significantly lower IFN-γ response compared with wild-type *L. major*. These data indicate that cysteine proteases of *L. mexicana* are critical in suppressing protective immune responses and that inhibition of CPB may prove to be a valuable immunomodulatory strategy for chronic forms of leishmaniasis. The Journal of Immunology, 2003, 171: 3711–3717.

*Leishmania mexicana*, like other New World Leishmania species of the *L. mexicana* complex, more often causes chronic lesions in human infections than *Leishmania major*, an Old World species that generally yields self-limited human disease (1). These differences are also seen following experimental infection in mice. Most mouse strains, such as C3H, C57BL/6 (B6), and 129, are resistant to *L. major*, but develop nonhealing lesions following infection with *L. mexicana* (2). The inability of these mouse strains to heal following infection with *L. mexicana* has been associated with an insufficient Th1 response, rather than a dominant Th2 response. The lack of an effective Th1 response is not due to a lack of IL-12, as treatment with exogenous IL-12 does not heal lesions caused by *L. mexicana* species (3). Instead, IL-12 responsiveness is low due to diminished T cell expression of a functional IL-12R following infection with the *L. mexicana* complex parasite, *Leishmania amazonensis*, as compared with *L. major* infection (4). These studies suggest that differences in expression of parasite-derived factors between *L. major* and *L. mexicana* may explain the differences in disease outcome. In this study, we investigated the role of a family of *L. mexicana*-derived cysteine proteases (CPs)4 in modulating host immunity.

Several pathogenic protozoan parasites, including *Entamoeba histolytica* (5), *Leishmania* (6–8), *Trypanosoma cruzi* (9), *Trypanosoma congoense* (10, 11), and *Trypanosoma brucei* (12), express multiple CP enzymes (reviewed in Ref. 13). *L. mexicana* possesses three types of CPs of the papain superfamily: CPA and CPB have homology to mammalian cathepsin L; CPC has homology to mammalian cathepsin B, but has substrate specificity closer to cathepsin L. CPB is especially abundant, and the 19 CPB genes are stage regulated, with most having the greatest expression in amastigotes (the parasite stage that occurs in the mammalian host). These enzymes have been localized to the megasomes (large extended lysosomes found in *L. mexicana*, but not *L. major*), but are also present in the parasitophorous vacuole, and in abundance in the extracellular tissue of murine leishmaniasis lesions (14). Although *L. major* contains a homologous array of CPB genes, CPB activity is considerably lower than in *L. mexicana* (15), which shows that there are major differences in enzyme levels and/or specificities between these two *Leishmania* species. Given the immunomodulatory roles of CPs in other protozoan infections (5, 16, 17), we hypothesized that the differential CPB activities might account for differences in the immunologic and pathologic responses to *L. mexicana* and *L. major*.

In this study, we examined the role of CPB in the immune response to *L. mexicana* by comparing infections of wild-type (WT) *L. mexicana* and *L. mexicana* lacking CPB (Δcpb) in C3H mice. Δcpb-infected C3H mice initially developed lesions comparable to WT *L. mexicana*-infected mice, with similar parasite burdens.

4 Abbreviations used in this paper: CP, cysteine protease; CPB, cysteine protease B protein; CPB, cysteine protease B gene; Δcpb, CPB-deficient *L. mexicana* mutant; FTAg, freeze-thaw Ag; L major/CPB, *L. major* transfected with CPB-containing cosmid pGL263; LN, lymph node; SLA, soluble leishmanial Ag; WT, wild type.
However, in contrast to WT *L. mexicana* infections, \( \Delta cpb \) infections induced a Th1 response and exhibited IL-12- and STAT4-dependent lesion resolution. In addition, expression of the *L. mexicana* *CPB* gene array in *L. major* was associated with a decreased Th1 response. These results indicate that the differences in CPB activity or specificity may help to explain the differences between the diseases caused by these two *Leishmania* species.

Materials and Methods

**Mice**

C3HeB/FeJ, C57BL/6, and B6 IL-12p40 \(^{-/-} \) mice (4–6 wk old) were obtained from The Jackson Laboratory (Bar Harbor, ME). J. Ihe (St. Jude Children’s Research Hospital, Memphis, TN) originally generously provided STATA \(^{+/-} \) mice, on a mixed 129Sv/C57BL/6 (129/B6) background. The 129/B6 control mice were randomly interbred alongside the STATA \(^{+/-} \) mice for similar numbers of generations. Mice (at least three per group) were age and sex matched for all experiments. Animals were maintained in a specific pathogen-free environment, and the animal colony was screened regularly for the presence of murine pathogens.

**Leishmania parasites and Ags**

Promastigotes of WT *L. mexicana* (MNYC/BZ/62/M379), a CPB-deficient clone from the same strain (\( \Delta cpb \)) (6); *L. major* (MHOM/IL/80/Friedlin); and *L. major* transfected with cosmid pGL263 containing the *L. mexicana* *CPB* gene array (\( L. major/CPB \)) were grown as previously described in Grace’s medium (Life Technologies/BRL, Grand Island, NY) (5). Stationary-phase promastigotes (day 7 of culture) were washed three times in PBS, and 5 \( \times \) 10\(^6 \) parasites were injected s.c. (50 \( \mu l \)) into the hind footpad of mice. Lesion size was followed using a dial caliper and expressed as the hind footpad thickness of mice. Won, V.B. (St. Louis, MO). WT *L. major* and WT *L. mexicana* failed to grow in the presence of 50 \( \mu g/ml \) hygromycin B (Sigma-Aldrich, St. Louis, MO). WT *L. major* and WT *L. mexicana* failed to grow in the presence of 50 \( \mu g/ml \) hygromycin B (Sigma-Aldrich, St. Louis, MO). WT *L. major* and WT *L. mexicana* failed to grow in the presence of 50 \( \mu g/ml \) hygromycin B (Sigma-Aldrich, St. Louis, MO).

**Parasite quantitation**

Parasite quantitation was performed by limiting dilution, as previously described (19), except that some assays involved 2-fold rather than 10-fold dilution series. Data from three to six samplings of the same footpad preparation were analyzed using a method developed by Finney (20), and means and SEM for two to five animals were calculated.

**In vitro restimulation**

Single-cell suspensions were prepared from the draining lymph nodes (LNs) or spleens and cultured, as described (21). Cells were stimulated with 10 \( \mu g/ml \) (10\(^7 \) cell equivalents/ml) (or concentrations as stated) of homologous FTAg (*L. mexicana* or *L. major*), or SLA (50 \( \mu g/ml \)). M1, an anti-IL-4R mAb (a generous gift from F. Finkelman, University of Cincinnati, Cincinnati, OH, and Immunex, Seattle, WA), was included at 2.5 \( \mu g/ml \), to increase the sensitivity of IL-4 detection. Cells were incubated for 3 days at 37\(^\circ\)C in a 5% CO\(_2\) incubator, and supernatants were assayed for IL-4 and IFN-\( \gamma \) by ELISA (21). Uninfected mice had no detectable IL-4 or IFN-\( \gamma \) production in response to FTAg or SLA in these experiments.

**Cytokines**

Murine IL-12 was a generous gift of S. Wolf and J. Syypek (Genetics Institute, Cambridge, MA).

**Transfection of *L. major* with pGL263**

pGL263, a cosmid bearing multiple *CPB* genes, was originally selected from an *L. mexicana* library by hybridization with probes derived from the unique 5’ and 3’ *CPB* gene array flanking sequences and the CPB protein coding sequence (22). *L. major* promastigotes were transfected with 5 \( \mu g \) of pGL263 cosmid DNA, and parasites were selected in complete modified Eagle’s medium (designated complete HOMEM medium), pH 7.5, with 10% (v/v) heat-inactivated FBS supplemented with hygromycin (50 \( \mu g/ml \)), as described previously (23). Individual clones were isolated on 1% (w/v) agar complete HOMEM plates, and then transferred to complete HOMEM medium. The cloned *L. major/CPB* was passed through BALB/c mice before reisolation for infection experiments.

**Gelatin SDS-PAGE CP analyses**

CPB enzymatic activity was assayed on gelatin SDS-PAGE, as previously described (24). Coomassie blue stain was used to visualize hydrolysis of 0.2% gelatin (Sigma-Aldrich) copolymerized in the 12% separating gel.

**Statistics**

When error bars are shown, data represent the mean \( \pm \) SEM for groups of individual mice. All \( p \) values shown are for unequal variance Student’s \( t \) tests.

**Results**

Enhanced control of *L. mexicana* \( \Delta cpb \) in C3H mice

C3H mice were infected with WT *L. mexicana* and \( \Delta cpb \), and the courses of infection were monitored. For comparison, some mice were also infected with *L. major*. In contrast to the nonhealing lesions seen in mice infected with WT *L. mexicana*, \( \Delta cpb \) infection was associated with the development of lesions that peaked at 6 wk and healed fully by 16 wk, similar to *L. major* lesions (Fig. 1A). Parasite burdens of WT *L. mexicana* and \( \Delta cpb \) were the same at 3 and 7 days postinfection, suggesting that in vivo \( \Delta cpb \) is able to survive in a manner similar to WT *L. mexicana* (Fig. 1B). By 2 wk, parasite burdens in \( \Delta cpb \) infection were over 3 logs lower than WT *L. mexicana* parasite burdens. Furthermore, \( \Delta cpb \) parasite burdens increased modestly from 3 days to 4 wk postinfection (\( p < 0.05 \)), confirming that these parasites can replicate in vivo. By 18 wk postinfection, parasite burdens were over 5 logs greater in WT *L. mexicana* than in \( \Delta cpb \) infection. Thus, \( \Delta cpb \) parasite burdens correlated with the healing of lesions. As with *L. major* infection (25), \( \Delta cpb \) infection did not lead to sterile immunity: low numbers of parasites were present 18 wk postinfection, which confirmed that \( \Delta cpb \) parasites can survive long-term in vivo (25).

Healing in \( \Delta cpb \) infection is associated with a protective Th1 response

To determine whether the ability of mice to heal following \( \Delta cpb \) infection is associated with the generation of an IFN-\( \gamma \)-dominated Th1 response, the immune responses of mice infected with WT *L. mexicana* were monitored. As before, \( \Delta cpb \) infection is associated with a decreased Th1 response compared to WT *L. mexicana* infection. In contrast to the nonhealing lesions seen in mice infected with WT *L. mexicana*, \( \Delta cpb \) infection was associated with the development of lesions that peaked at 6 wk and healed fully by 16 wk, similar to *L. major* lesions (Fig. 1A). Parasite burdens of WT *L. mexicana* and \( \Delta cpb \) were the same at 3 and 7 days postinfection, suggesting that in vivo \( \Delta cpb \) is able to survive in a manner similar to WT *L. mexicana* (Fig. 1B). By 2 wk, parasite burdens in \( \Delta cpb \) infection were over 3 logs lower than WT *L. mexicana* parasite burdens. Furthermore, \( \Delta cpb \) parasite burdens increased modestly from 3 days to 4 wk postinfection (\( p < 0.05 \)), confirming that these parasites can replicate in vivo. By 18 wk postinfection, parasite burdens were over 5 logs greater in WT *L. mexicana* than in \( \Delta cpb \) infection. Thus, \( \Delta cpb \) parasite burdens correlated with the healing of lesions. As with *L. major* infection (25), \( \Delta cpb \) infection did not lead to sterile immunity: low numbers of parasites were present 18 wk postinfection, which confirmed that \( \Delta cpb \) parasites can survive long-term in vivo (25).

**FIGURE 1.** Enhanced control of *L. mexicana* \( \Delta cpb \) in C3H mice. A, C3H mice were infected in the right hind footpad with 5 \( \times 10^6 \) stationary-phase WT *L. mexicana* (WT *L. mex*), \( \Delta cpb \), and *L. major* promastigotes, and lesion size was monitored. After 5 wk, WT *L. mexicana* lesions were larger than \( \Delta cpb \) lesions, \( p < 0.02 \). B, At the indicated times, mice were sacrificed and footpad lesion parasite burdens were determined by limiting dilution. \( * \), \( p < 0.05 \) for the difference between WT *L. mex* and \( \Delta cpb \). This is a representative experiment of three with similar results.
mexicana and Δcpb were assessed. Cells from Δcpb-infected mice produced more IFN-γ than cells from WT L. mexicana-infected mice at 4 wk postinfection (Fig. 2A). Furthermore, addition of IL-12 during restimulation substantially increased the IFN-γ response of cells from Δcpb-infected mice, but had no effect on cells from WT L. mexicana-infected mice. Greater IFN-γ responses were seen as early as 3 days postinfection with ~3.5 times more IFN-γ in Δcpb infection than WT L. mexicana infection, at two Ag concentrations (Fig. 2B). By 18 wk postinfection, draining LNs were very small, and responses had waned, but splenic IFN-γ responses were still increased in Δcpb as compared with WT L. mexicana infection (3.7 vs 0.7 ng/ml, in response to Ag). This indicates that healing of Δcpb infection is associated with an increased immune response, characterized by elevated IFN-γ production. Thus, CPB appears to be involved in suppression of a protective immune response.

**Vaccination with Δcpb protects C3H mice from WT L. mexicana challenge**

To determine whether the immune response induced by Δcpb is sufficient to protect mice from challenge with the more virulent WT L. mexicana parasite, C3H mice that had healed Δcpb infection were challenged at 12 wk with WT L. mexicana promastigotes (Fig. 3). These mice were protected from challenge as compared with the typical chronic disease seen in naive animals with L. mexicana infection. These data suggest that CPB most likely blocks initiation of a protective immune response, but is unable to suppress an established one.

**IL-12p40−/− mice fail to control Δcpb infection**

Unlike L. major infection, in which IL-12 is essential to prevent progressive disease, IL-12 does not play this role in L. mexicana infection, as B6 IL-12p40−/− (p40−/−) mice have the same controlled, chronic lesions as B6 mice, possibly due to other cytokines such as type I IFNs, IL-27, or IL-2 signaling through STAT4 (3). We therefore investigated whether IL-12 is also dispensable in Δcpb infection. We infected p40−/− and B6 mice with Δcpb and found that p40−/− mice developed progressive disease (Fig. 4A). By 48 wk postinfection with Δcpb, parasite burdens were nearly 5 orders of magnitude greater in p40−/− mice than in control B6 mice (Fig. 4B). This demonstrates that an IL-12-mediated Th1 immune response is required for control of Δcpb parasite numbers and to heal lesions, and provides compelling evidence that CPB actively manipulates host immune responses. As expected, IFN-γ responses were lower, and IL-4 responses higher in p40−/− mice as compared with B6 control mice (Fig. 4, C and D). These experiments also show that CPB acts to inhibit a step in the protective pathway involving, or perhaps upstream of, the IL-12 signaling pathway.

**STAT4−/− mice fail to control Δcpb infection**

In order to strengthen the finding that healing of Δcpb infection is in fact due to a Th1 immune response rather than to nonimmunologic factors, mice lacking the transcription factor STAT4 were...
infected with Δcpb parasites. STAT4−/− mice, which are deficient in Th1 development (26, 27), were highly susceptible to WT L. mexicana infection, and lesion size was monitored. After 16 wk, Δcpb-infected STAT4−/− mice had larger lesions than 129/B6 mice (p < 0.05). B, At 44 wk postinfection, Δcpb-infected mice were sacrificed and footpad lesion parasite burdens were determined by limiting dilution. WT L. mexicana infection was terminated early due to large lesion sizes. *, p < 0.02. At 44 wk postinfection, draining LN cells from Δcpb-infected mice were incubated with and without FTAg (Ag, 10 μg/ml) ± murine rIL-12, and supernatants were assayed for IFN-γ (C) and IL-4 (D) by ELISA. This is a representative experiment of two with similar results.

FIGURE 5. STAT4−/− mice fail to control Δcpb infection. A, STAT4−/− (knockout (KO)) and control 129/B6 (WT) mice were infected as in Fig. 1 with Δcpb and WT L. mexicana (L. mex), and lesion size was monitored. After 16 wk, Δcpb-infected STAT4−/− mice had larger lesions than 129/B6 mice (p < 0.05). B, At 44 wk postinfection, Δcpb-infected mice were sacrificed and footpad lesion parasite burdens were determined by limiting dilution. WT L. mexicana infection was terminated early due to large lesion sizes. *, p < 0.02. At 44 wk postinfection, draining LN cells from Δcpb-infected mice were incubated with and without FTAg (Ag, 10 μg/ml) ± murine rIL-12, and supernatants were assayed for IFN-γ (C) and IL-4 (D) by ELISA. This is a representative experiment of two with similar results.

IFN-γ responses from in vitro restimulation showed barely detectable levels of IFN-γ in STAT4−/− mice in response to Ag, with or without IL-12 (Fig. 5C). IL-4 responses were not significantly different in Δcpb-infected STAT4−/− and 129/B6 control mice (Fig. 5D). These data support the role of a Th1 response in controlling parasite burdens and lesion growth.

Expression of multiple L. mexicana CPB genes in L. major leads to an early suppression of IFN-γ production

To definitively demonstrate that CPB is responsible for immune suppression, we transfected L. major with the L. mexicana CPB gene array on a drug-selectable cosmid (pGL263) (Fig. 6A). This cosmid has been shown to partially restore virulence when expressed in Δcpb parasites (22). An enzyme activity gel demonstrates that stationary-phase promastigotes of the L. major transfectant (L. major/(CPB)) express greatly increased CPB activity compared with the trace amounts seen in WT L. major (Fig. 6B). To test the influence of L. mexicana CPB expression in L. major on the immune response, C3H mice were infected with WT L. major and L. major/(CPB). An analysis of the effects of L. mexicana CPB expression in L. major on the long-term course of infection was not possible due to the loss of the cosmid beginning at 4 wk. However, at 3 days postinfection, draining LN cells from WT L. major-infected C3H mice produced significantly more IFN-γ than those from L. major/(CPB)-infected mice (3-fold increase), when stimulated with a wide range of parasite Ag doses (Fig. 7A). Mean IL-4 responses were not different between WT L. major and L. major/(CPB) (data not shown). At 3 days postinfection, parasite burdens were significantly higher (by 8-fold) in L. major/(CPB) than WT L. major infection, showing that early suppression of IFN-γ may already affect parasite survival (Fig. 7B).

Discussion

C3H mice are able to heal L. major infection with a Th1 response, but maintain chronic nonhealing lesions with L. mexicana infection (2, 28, 29). The reasons for this have remained unclear. In this study, we discovered that infection of C3H mice with L. mexicana lacking all CPB genes (Δcpb) leads to healing and an associated decrease in parasite burden, with kinetics very similar to that of L.
major infection. IFN-γ production was enhanced by the deletion of CPB genes. We also found that this immunity is long-lived and cross protects against WT L. mexicana challenge. Furthermore, when multiple L. mexicana CPB genes were transfected into L. major, the early IFN-γ response was diminished. L. major possesses genes homologous to the L. mexicana CPB gene array (30), but L. major lacks the abundant CP activity seen with L. mexicana. This indicates that either CPB expression levels or substrate specificities of the enzymes are quite different between the New World L. mexicana and Old World L. major. Levels of expression and/or specificities of the many CPBs of L. mexicana have recently been shown to be very important in virulence, as re-expression of multiple CPB genes from the pGL263 cosmid, but not a single CPB gene, partially restored virulence of Δcpb in BALB/c mice (22). Together these findings show that CPB has a role in suppressing the Th1 immune response to L. mexicana, and the differences between CPB expression in L. major and L. mexicana may help to explain differences in disease outcomes.

The present studies and previous work on Δcpb have shown that CPB enzymes are important virulence factors. Δcpb infection is associated with lower parasite burdens and the development of a Th1 response, with full healing of lesions in C3H mice and quite attenuated growth in BALB/c mice. However, until now it has not been clear whether the immune response is responsible for these findings. Poor parasite growth or survival could lead to the development of a Th1 response and healing of lesions. In fact, although Δcpb promastigotes multiply in culture with similar kinetics to WT L. mexicana, and both efficiently differentiate into axenic amastigote-like forms (6), it appears that Δcpb promastigotes survive in only a subset of macrophages (6, 31). However, we have now shown that in C3H mice Δcpb promastigotes transform into amastigotes and survive for the first 7 days of infection equally well as WT L. mexicana. This is in agreement with the finding that Δcpb amastigotes do not have a defect in macrophage infection and survival in vitro (31), and also suggests that perhaps promastigotes may not have a major survival defect in vivo. Moreover, infections of IL-12p40−/− and STAT4−/− mice showed that in the absence of a Th1 response, Δcpb is capable of causing progressive disease with very high parasite burdens (with increases greater than 5 orders of magnitude). Thus, the immune response is crucial for parasite control and explains the drop in parasite burdens after 2 wk or more postinfection in C3H mice. Lesion growth in STAT4−/− and p40−/− mice was faster in WT L. mexicana vs Δcpb infection, showing that even in the absence of a Th1 response Δcpb parasites may be somewhat compromised. Although this suggests that inherent differences in these parasites play some role in the healing of Δcpb infection, the ultimately progressive disease seen in p40−/− and STAT4−/− mice demonstrates a clear dependence on Th1 immunity for healing of Δcpb infection.

CPs are found in a wide variety of organisms from bacteria to plants and mammals. They have been shown to be important virulence factors in a variety of parasitic organisms (32). The CPs of kinetoplastid protozoa in particular, including L. mexicana, have been studied by several groups. The CPB genes of L. mexicana are stage-regulated cathepsin L-like enzymes, with 2 of the 19 genes in the array being expressed in infective metacyclic promastigotes, while the other 17 are predominantly expressed in amastigotes (7); thus, expression occurs exclusively in parasite stages that interact with the mammalian host. Although these CPB enzymes are found in the megasomes (extended, large lysosomes), they have been found in the parasitophorous vacuole and extracellularly in lesions (14), giving the enzymes ample opportunity to interact with host proteins.

The current findings, that L. mexicana CPB enzymes are virulence factors because they suppress the host immune responses, open the door to three important areas of research: investigation of the mechanisms of CPB suppression of Th1 responses, explanation for the parasite species differences between Leishmania disease pictures, and development of novel immunomodulatory therapeutic approaches to parasite infections.

The mechanisms by which CPB enzymes suppress a protective Th1 response are not well understood. Papain family CPs such as papain itself (33), schistosome Ags (34), house dust mite Der p 1 (35, 36), cruzipain from T. cruzi (37), and purified CPB2.8 from L. mexicana can induce Th2 responses (38). In several of these systems, including the L. mexicana CPB enzyme, this immune deviation required enzymatic activity and was not a passive Ag role. The role of CPB in inducing Th2 responses, however, is strongest in BALB/c mice that are prone to Th2 deviation (39). In the experiments presented in this work, IL-4 production in in vitro stimulation from L. mexicana-infected C3H mice was barely detectable, even in the presence of M1 (anti-IL-4R Ab). Also, B6 IL-4−/− and 129/B6 STAT6−/− mice had chronic footpad lesions when infected with L. mexicana species (4) (L. Buxbaum, unpublished results), questioning the dominant role of Th2 responses in leading to chronic disease in the more resistant strains of mice (C3H, B6, 129/B6). Therefore, we do not believe that the major role of CPB in L. mexicana infection in these mouse strains is related to IL-4.

CPB may suppress a healing Th1 response nonspecifically by suppression of Ag presentation or T cell proliferation, directly by altering Th1 pathways, or through effects on crucial transcription factors. L. amazonensis (an L. mexicana complex parasite) has been shown to take up and cleave MHC class II from the host parasitophorous vacuolar membrane (40), which may lead to the down-regulation of Ag presentation of parasite proteins (41, 42). This digestion of MHC class II may involve CPB. At high concentrations, L. mexicana CPB2.8 and dust mite Der p 1 can cleave CD25 on T cells (38, 43). Cleavage of CD25 (a component of the IL-2R) could decrease T cell proliferation, and would explain the minimal cytokine responses seen in C3H mice to L. mexicana infection. Alternatively, CPB may play a role in the function of immunosuppressive factors, such as IL-10 and TGF-β. A purified CP from Leishmania chagasi, as well as parasite extract, can activate human TGF-β (44), a known suppressive cytokine in Leishmania infection (45, 46). A CP from the Gram-negative bacteria Porphyromonas gingivalis has been shown to cleave IL-12 and thus directly inhibit Th1 development and IFN-γ production (47). For this to be important in L. mexicana infection, exogenous IL-12, as well as endogenous IL-12, would have to be cleaved efficiently by CPB, as exogenous IL-12 treatment failed to resolve chronic lesions in L. mexicana species infections of C3H mice (3, 4). Recent experiments have shown that L. mexicana CPB may cleave the transcription factor NF-κB and its regulatory partner IκB with a number of possible immunosuppressive effects on infected macrophages (P. Cameron, unpublished results). Thus, there are several potential mechanisms for the role of CPB in suppression of protective immunity, although the important substrates and pathways are not yet clear.

There is now growing evidence that L. major and L. mexicana, which diverged evolutionarily 80 million years ago (48), have also diverged with respect to the importance of virulence factors. As already mentioned, cytokines such as IL-4 and IL-12 appear to play different roles in the immune responses to these parasites. Furthermore, the disease courses in more resistant mouse strains (e.g., C3H and B6) are quite different for L. major infection, in which lesion resolution occurs, and L. mexicana infection, in...
which chronic lesions persist. Lipophosphoglycan and other phosphoglycan molecules are important \textit{L. major} virulence factors, but do not play this role for \textit{L. mexicana} (49, 50). Likewise, the protein \textit{Leishmania} homolg of receptors for activated C kinase is an immunodominant Ag responsible for virulence of \textit{L. major} in BALB/c mice, but not for \textit{L. mexicana} (51, 52). The current study helps support the idea that CPB enzymes of \textit{L. mexicana} also represent important virulence factors that are parasite species specific and may help to explain the different disease outcomes.

CP inhibitors offer interesting possibilities for new therapeutic approaches against parasitic organisms. Specific CP inhibitors not only can kill \textit{L. major} in vitro, but also can cure \textit{L. major} infection in BALB/c mice (53). In fact, inhibitors of CPs from \textit{T. cruzi} (54), \textit{B. brucei} (55), and \textit{Plasmodium} (56, 57) have demonstrated efficacy in preliminary animal studies, and KI1777, a CP inhibitor of the \textit{T. cruzi} enzyme cruzipain, will enter clinical trials for Chagas disease very soon (52). It is also interesting that CP inhibitors such as N-benzoxylcarbonyl-\textit{D}-aladiazomethylketone, which do not directly kill \textit{L. mexicana} axenic (cultured) amastigotes in liquid culture, do induce the killing of parasites in infected macrophages, supporting a role for CPs in suppression of host defense (6). Thus, CPs hold promise as therapeutic targets for a wide range of infections by thwarting the immune-suppressive effects of these enzymes, as well as by the more traditional mechanism of direct parasite killing (13, 32).

Acknowledgments

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References