Captopril Prevents Experimental Autoimmune Myocarditis

Lisa M. Godsel, Juan S. Leon, Kegiang Wang, Jamie L. Fornek, Agostino Molteni and David M. Engman

*J Immunol* 2003; 171:346-352; doi: 10.4049/jimmunol.171.1.346

http://www.jimmunol.org/content/171/1/346

---

**References**
This article cites 63 articles, 17 of which you can access for free at:

http://www.jimmunol.org/content/171/1/346.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
Captopril Prevents Experimental Autoimmune Myocarditis

Lisa M. Godsel, Juan S. Leon, Kegiang Wang, Jamie L. Fornek, Agostino Molteni, and David M. Engman

Captopril, an angiotensin-converting enzyme inhibitor, is widely used in the treatment of a variety of cardiomyopathies, but its effect on autoimmune myocarditis has not been addressed experimentally. We investigated the effect of captopril on myosin-induced experimental autoimmune myocarditis. A/J mice, immunized with syngeneic cardiac myosin, were given 75 mg/L of captopril in their drinking water. Captopril dramatically reduced the incidence and severity of myocarditis, which was accompanied by a reduction in heart weight to body weight ratio and heart weight. Captopril specifically interfered with cell-mediated immunity as myosin delayed-type hypersensitivity (DTH) was reduced, while anti-myosin Ab production was not affected. Captopril-treated, OVA-immunized mice also exhibited a decrease in OVA DTH. In myosin-immunized, untreated mice, injection of captopril directly into the test site also suppressed myosin DTH. Interestingly, captopril did not directly affect Ag-specific T cell responsiveness because neither in vivo nor in vitro captopril treatment affected the proliferation, IFN-γ secretion, or IL-2 secretion by Ag-stimulated cultured splenocytes. These results indicate that captopril ameliorates experimental autoimmune myocarditis and may act, at least in part, by interfering with the recruitment of cells to sites of inflammation and the local inflammatory environment. The Journal of Immunology, 2003, 171: 346–352.

Myocarditis, inflammation of the heart, is characterized by myocyte necrosis and degeneration with mononuclear cell infiltration in the presence or absence of fibrosis (1). In the U.S., ~2500 people develop myocarditis each year (2, 3), although the prevalence of this disease is probably underestimated (4, 5). Myocarditis may be caused by infections with bacteria, viruses, parasites, and fungi, as well as by drugs and toxins. Virally induced myocarditis is commonly caused by coxsackievirus infection in North America and Europe, and parasite-induced myocarditis is commonly caused by Trypanosoma cruzi infection in Latin America (1, 3, 5–7).

Treatments for myocarditis are directed toward reducing or eliminating the infectious agent and associated disease complications, such as congestive heart failure, cardiogenic shock, conduction abnormalities, dysrhythmias, and thromboembolism (8). These complications are typically treated with diuretics, digitalis, β blockers, and vasodilators, such as angiotensin II receptor antagonists and angiotensin-converting enzyme (ACE) inhibitors (9). One commonly prescribed ACE inhibitor is captopril, which binds to ACE via its peptide-binding pocket and inhibits the functions of ACE, particularly the formation of angiotensin II from angiotensin I and the breakdown of bradykinin (10). Among the many ACE inhibitors and angiotensin II receptor antagonists, captopril has been shown to modulate chemotaxis, motility, adhesion, differentiation, activation, and cytokine and chemokine production of immune cells (reviewed in Ref. 11). Captopril is effective at ameliorating many human cardiomyopathies (12, 13), although its direct effect on human myocarditis has not been addressed. The drug decreases inflammation, calcification, and fibrosis in several models of infectious myocarditis, including encephalomyocarditis virus (14–16), coxsackievirus B3 (17–19), and T. cruzi (20). However, the effect of captopril on experimental models of Ag-induced autoimmune myocarditis has not been addressed.

An established model of experimental autoimmune myocarditis (EAM) is induced in susceptible strains of mice upon immunization with the α H chain of cardiac myosin (21). EAM is histologically similar to human myocarditis, with myocyte swelling and necrosis accompanied by mononuclear cell infiltration and fibrosis. Studies have shown that EAM is a T cell-mediated disease, requiring both CD4+ and CD8+ subsets (22–26). B cells are not vital for Ag presentation in EAM, and autoantibodies are not necessary for the progression of myocarditis (22, 27, 28).

To study the effect of ACE inhibition on the development of autoimmune myocarditis, we administered captopril to A/J mice immunized with cardiac myosin. We found that captopril ameliorates myocarditis and decreases cell-mediated inflammatory responses found in EAM. These results demonstrate that captopril treatment is indeed an effective method for inhibiting autoimmune myocarditis, and although it decreases cell-mediated immune responses, it does not directly affect T cell function.

Materials and Methods

Experimental animals

Male A/J mice (The Jackson Laboratory, Bar Harbor, ME) were 6–8 wk of age at initiation of the experiments. DO11.10 BALB/c mice were a gift from S. Miller (Northwestern University, Chicago, IL). Mice were anesthetized by a single i.p. injection of 60 mg/kg sodium pentobarbital for each experimental manipulation. The use and care of mice were conducted in accordance with the guidelines of the Center for Comparative Medicine at Northwestern University.
Preparation of myosin
Cardiac myosin H chains were purified according to the method of Shive erick et al. (29), with modifications as described (30).

Induction of autoimmune myocarditis
Mice were immunized with myosin (300 μg) in an emulsion of CFA (Difco, Detroit, MI) in a total volume of 0.1 ml. Mice received s.c. injections in three sites in the dorsal flank. Seven days later, mice were boosted in an identical manner.

OVA immunization
Mice were immunized with an emulsion of OVA (100, 75, 50, or 25 μg; Sigma-Aldrich, St. Louis, MO) in CFA in a total volume of 0.1 ml. Mice received s.c. injections in three sites in the dorsal flank. Seven days later, mice were boosted in an identical manner.

Captopril treatment regimen
Mice were given drinking water containing or lacking 75–100 μg/ml captopril from the day of immunization through the day of sacrifice. We tested a variety of doses of captopril and chose for the actual experiment the highest dose that gave a decrease in myosin-induced myocarditis without mortality. Administration of captopril at concentrations greater than 120 μg/ml in the water led to excessive mortality. The range of doses tested was chosen based on other reports (15, 31), with 2 mg/ml as the upper end dose (32). The amount of water consumed and the weights of the mice were monitored and the amount of captopril was adjusted so that the mice received ~25 mg captopril/kg body weight/day. Captopril stimulated water consumption in our mice, as reported (33), to 5–7 ml per mouse per day throughout the course of treatment.

Serum ACE activity
Serum ACE activity was determined by the spectrophotometric method of Cushman and Cheung (34) using the synthetic substrate hippuryl-L-histidyl-L-leucine. Serum was treated with HCl and ethyl acetate and dehydrated. Hippuryl-L-histidyl-L-leucine content was then determined using a biuret method utilizing BSA as a standard. ACE levels were expressed as milliunits per ml of serum. The increase in serum ACE correlates with the presence of captopril due to the deregulation of a negative feedback loop in the renin-angiotensin system.

Histologic evaluation of disease
Hearts were excised, fixed in 10% buffered Formalin, and embedded in paraffin. Four sections per heart were stained with H&E and Masson’s trichrome and examined by light microscopy. Masson’s trichrome was used to determine the extent of collagen deposition as an indicator of fibrosis. Each section was examined blinded for evidence of mononuclear cellular inflammation, necrosis and mineralization, and fibrosis, and was assigned a histologic score between 0 (no involvement noted) and 4 (100% involvement), with 1, 2, and 3 representing 25, 50, and 75% involvement of the histologic section. Independent blinded observers obtained substantial (0.60–0.79) to almost perfect (0.80–1.00) agreement on their scoring by weighted κ statistic (35), as measured on a representative sample of 20 heart sections: overall agreement (0.82), inflammation (0.80), necrosis (0.96), and fibrosis (0.92).

Measurement of Ag-specific delayed-type hypersensitivity
Myosin- and OVA-specific delayed-type hypersensitivity (DTH) was quantitated using a standard ear-swelling assay (30). Ag-induced ear swelling was the result of mononuclear cell infiltration and exhibited typical DTH kinetics (i.e., minimal swelling at 4 h, maximal swelling at 24–48 h postinjection).

Serologic analysis
Myosin- and OVA-specific ELISAs were used to analyze the levels and isotype specificities of the Ag-specific Abs, as described (36). Each serum sample was analyzed separately, and the data were compiled for each group. Each plate was also tested with sera from one particular mouse immunized with myosin/CFA, a second immunized with OVA/CFA, and a third immunized with saline/CFA as controls.

In vitro T cell proliferation and cytokine assays
Spleens were forced through 100-mesh stainless steel screens to yield single cell splenocyte suspensions. RBCs in the spleen preparations were lysed by hypotonic shock in Tris-NH4Cl (pH 7.3), and the cells were washed and resuspended in HBSS (Life Technologies, Grand Island, NY). Cells were cultured in 96-well microtiter plates (Corning-Costar, Acton, MA) at 5 × 10^3 cells/well in DMEM containing 5 × 10^5 M 2-ME, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 0.1 M nonessential amino acids, and 5% FCS (HyClone, Logan, UT) in the presence of 10 μM OVA, 10 μM myosin, or medium alone as a negative control, and anti-murine CD3 (500 ng/ml, 2C11 clone; a gift from W. Karpus, Northwestern University) as a positive control. Cells were incubated at 37°C in a humidified atmosphere containing 5% CO2. The cells were pulsed with 1 μCi of [3H]Tdr (ICN Radiochemicals, Irvine, CA) after 72 h and harvested after 96 h, and [3H]Tdr uptake was detected using a Topcount microplate scintillation counter (Packard Instruments, Meriden, CT). Results are presented as the mean ± SEM of triplicate wells. Assessment of cytokine production was determined from lymph node culture supernatants harvested following 48-h stimulation with 10 μM OVA, 10 μM myosin, or medium alone as a negative control, and anti-CD3 as a positive control. The supernatants were tested for the presence of IL-2, IL-4, and IFN-γ by commercial ELISA kits (Endogen, Cambridge, MA). The detection limit of the cytokine ELISA kits is as follows: IL-2, 15.6 pg/ml; IL-4, 31.3 pg/ml; and IFN-γ, 48.8 pg/ml.

Statistical evaluation
All values are expressed as mean ± SEM. The statistical significance of DTH, T cell proliferations, cytokine levels, and Ab isotypes was analyzed by one-way ANOVA, followed by a two-tailed t test and post hoc Bonferroni analysis. The statistical significance of disease incidence and comparison of histologic scores were analyzed by Pearson’s χ2. Agreement of blinded observer histopathology scores was analyzed by weighted κ. Mean histologic scores ± SD, although statistically invalid, will be provided to aid the reader and to continue the convention of other autoimmune myo- carditis papers. Values of p < 0.05 were considered significant.

Results
Captopril increases serum ACE levels
To study the effect of capto pril on autoimmune-mediated inflammation and fibrosis, we added captopril to the drinking water of myosin-immunized and saline-immunized AJ mice. Each capto- pril-treated mouse received 25 mg drug/kg body weight per day. We confirmed that the capto pril treatment was effective by assaying for increased levels of ACE in treated mice at day 21 postimmunization. Increased ACE levels result from the capto pril-induced negative feedback loop of the renin-angiotensin system in which a decrease of angiotensin II stimulates the production of ACE (34). Captopril-treated myosin and saline-immunized mice had significantly (p < 0.001) higher levels of ACE than did untreated controls (Fig. 1). These results indicate that capto pril was
Captopril prevents myocarditis in myosin-immunized mice. At 21 days postimmunization, hearts from the four groups described in Fig. 1 were analyzed grossly (Gross) and histopathologically by staining with H&E or Masson’s trichrome (Trichrome). Representative hearts and tissue sections are shown. Hearts from saline-immunized mice were indistinguishable from hearts from saline-immunized, captopril-treated mice (PBS/CFA + Captopril).

Captopril reduces cardiac hypertrophy in myosin-immunized mice

Twenty-one days postimmunization, gross analysis of the hearts of captopril-treated mice revealed a significant decrease in the typical signs of myocarditis: hypertrophy, induration, and pallor. Captopril-treated, myosin-immunized mice resembled saline-immunized mice (Fig. 2). Hypertrophy was reduced in treated mice, as evidenced by body weight and heart weight measurements (Table I). Captopril significantly reduced the heart weight, body weight, and heart weight to body weight ratios in myosin-immunized mice. Interestingly, both the heart weight and the heart weight to body weight ratios of myosin-immunized, captopril-treated mice were equivalent to those of saline-immunized mice. Captopril did not significantly affect the heart weight or heart weight to body weight ratio in saline-immunized mice, suggesting that the reduction of these parameters in myosin-immunized, captopril-treated mice was due to reduction in hypertrophy.

Captopril ameliorates myocarditis in myosin-immunized mice

To investigate whether the reduction of hypertrophy reflected a lack of tissue inflammation, we performed histopathologic analysis of hearts excised from myosin- and saline-immunized mice 21 days postimmunization. Captopril significantly reduced the incidence (Table I) and severity (Fig. 2, Table II) of myocarditis in myosin-immunized mice. Specifically, captopril reduced inflammation, fibrosis, and necrosis in these mice. Restricting the analysis to only diseased hearts showed that disease severity in affected mice was reduced, although not to a statistically significant level (Table II).

Captopril reduces Ag-specific DTH

We hypothesized that the reduction in cardiac inflammation in captopril-treated, myosin-immunized mice was due to an effect on myosin-specific immunity, which mediates cardiac inflammation in these mice (36). We assayed myosin-specific cellular immunity and found that captopril significantly reduced myosin DTH in myosin-immunized mice (Fig. 3c). These results suggest that captopril affects T cell-mediated inflammation in vivo, explaining the reduction of inflammation and damage observed in hearts of the captopril-treated, myosin-immunized mice. To determine whether the reduction of DTH by captopril was due to a suppression of T cell responses and not due to a reflection of captopril’s cardioprotective role (i.e., less myocardial damage results in decreased myosin autoimmune response (21)), we assayed OVA DTH in OVA-immunized mice treated with captopril. Captopril significantly reduced OVA DTH in OVA-immunized mice (Fig. 3c). These results suggest that captopril can reduce inflammatory responses to both a self and foreign Ag, irrespective of myocardial damage. Captopril has been shown to reduce local inflammatory processes

Table I. Captopril treatment decreases disease incidence, heart weight, and heart weight to body weight ratio in mice with experimental autoimmune myocarditis

<table>
<thead>
<tr>
<th>Group</th>
<th>Disease Incidence</th>
<th>Heart Weight (g)</th>
<th>Body Weight (g)</th>
<th>Heart Weight to Body Weight Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myosin/CFA + Captopril</td>
<td>6/30 (20%)*</td>
<td>0.087 ± 0.016**</td>
<td>21.1 ± 1.76**</td>
<td>3.97 × 10^{-3} ± 1.03 × 10^{-4}***</td>
</tr>
<tr>
<td>Myosin/CFA</td>
<td>21/33 (64%)</td>
<td>0.110 ± 0.023***</td>
<td>22.8 ± 2.49</td>
<td>4.83 × 10^{-3} ± 1.65 × 10^{-4}***</td>
</tr>
<tr>
<td>PBS/CFA + Captopril</td>
<td>0/11</td>
<td>0.084 ± 0.008</td>
<td>21.0 ± 2.22</td>
<td>4.03 × 10^{-3} ± 2.95 × 10^{-4}***</td>
</tr>
<tr>
<td>PBS/CFA</td>
<td>0/10</td>
<td>0.095 ± 0.011</td>
<td>22.8 ± 1.88</td>
<td>4.15 × 10^{-3} ± 2.86 × 10^{-4}***</td>
</tr>
</tbody>
</table>

*, p = 0.001 compared with myosin/CFA.

**, p < 0.05 compared with myosin/CFA.

***, p < 0.01 compared with myosin-immunized mice.

Table II. Captopril treatment decreases inflammation, necrosis, and fibrosis in mice with experimental autoimmune myocarditis

<table>
<thead>
<tr>
<th>Disease</th>
<th>Scores*†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>All Mice</td>
<td></td>
</tr>
<tr>
<td>Inflammation</td>
<td>+</td>
</tr>
<tr>
<td>Necrosis</td>
<td>−</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>−</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>−</td>
</tr>
</tbody>
</table>

| Affected Mice|   |   |   |   |   |       |          |
| Inflammation | + | 0 | 5 | 1 | 0 | 0 | 6 | 1.2 ± 0.4 |
| Necrosis     | − | 2 | 8 | 7 | 2 | 0 | 19 | 1.5 ± 0.8 |
| Fibrosis     | − | 4 | 10 | 5 | 0 | 0 | 19 | 1.1 ± 0.7 |
| Fibrosis     | − | 2 | 3 | 1 | 0 | 0 | 6 | 0.8 ± 0.8 |
| Fibrosis     | − | 1 | 11 | 7 | 0 | 0 | 19 | 1.3 ± 0.6 |

* Histologic scores were assigned by blinded observers and ranged from 0 (no involvement noted) to 4 (100% involvement), with 1, 2, and 3 representing 25, 50, and 75% involvement of the histologic section. The number of mice and the percentage of mice in the group receiving that score are given. Mice injected with saline (n = 8) or mice injected with saline and treated with captopril (n = 10) received scores of 0 for all parameters. The severity score is presented as the average ± SD.

†, p < 0.05 compared with untreated myosin-immunized mice.

‡‡‡, p < 0.01 compared with untreated myosin-immunized mice.
Captopril does not affect Ag-specific Ab production

Prevention of myocarditis in myosin-immunized mice by restoring peripheral T cell tolerance to myosin is associated with a decrease in myosin-specific Ab production (36). Moreover, other studies suggest that captopril decreases bulk Ab levels (46, 47). We investigated whether captopril treatment affected humoral responses to myosin and OVA at day 21 postimmunization, and found that myosin-specific and OVA-specific Ab production was not signifi-

Ag-specific T cell proliferation and cytokine secretion are not affected in vivo or in vitro by captopril administration

To determine whether the in vivo effects of captopril or Ag-specific cellular immunity are due to direct effects on T cell function, we measured the effect of captopril treatment in vitro on the Ag-specific stimulation of splenocytes derived from DO11.10 TCR transgenic mice in which the majority of T lymphocytes are specific for OVA. Splenocytes from untreated DO11.10 cultured with OVA exhibited strong proliferation and cytokine secretion in the presence or absence of captopril in the culture medium (Fig. 5). Splenocytes did not respond to myosin, a negative control. This prompted us to test splenocytes from captopril-treated OVA-immunized BALB/c mice. Splenocytes from mice receiving captopril for 21 days showed normal in vitro proliferation and cytokine secretion when stimulated by OVA or anti-CD3 (Fig. 6). These results suggest that captopril does not directly affect Ag-specific T cell responses.

Discussion

Captopril ameliorated autoimmune myocarditis, as evidenced by a reduction in cardiac hypertrophy and the incidence and severity of inflammation, necrosis, and fibrosis. Captopril also reduced in vivo cell-mediated inflammatory responses, as measured by a reduction of myosin- and OVA-specific DTH in Ag-immunized mice. This reduction in cell-mediated immunity by captopril was not due to a direct effect on T cells because these cells proliferated normally and secreted normal amounts of proinflammatory cytokines when necessary for the induction of DTH responses, including: deposition of extravasated fibrin (17), production of proinflammatory cytokines (40–42), recruitment of leukocytes (43, 44), and regulation of the renin-angiotensin system in dendritic cell function (45).

Therefore, we tested whether captopril could suppress DTH locally or required systemic administration. In myosin-immunized, untreated mice, local injection of captopril into the DTH site also significantly decreased myosin DTH compared with controls (Fig. 3b). These results indicate that captopril can act locally to mediate its effects and that its reduction of DTH may be due to a direct effect on T cell function, trafficking of T cells, or a generalized suppression of the inflammatory environment.

Captopril does not affect Ag-specific Ab production

Prevention of myocarditis in myosin-immunized mice by restoring peripheral T cell tolerance to myosin is associated with a decrease in myosin-specific Ab production (36). Moreover, other studies suggest that captopril decreases bulk Ab levels (46, 47). We investigated whether captopril treatment affected humoral responses to myosin and OVA at day 21 postimmunization, and found that myosin-specific and OVA-specific Ab production was not signifi-

![Image](https://example.com/image.png)
were assayed for the presence of IL-2 or IFN- 

B

wells. Error bars represent SEM.

pooled from at least

fi

osin), as a negative control, in the presence of varying concentrations of
cultures with 10 

myosin. Splenocytes were pooled from at least five mice. Symbols represent the mean of triplicate wells. Error bars represent SEM.

removed from the animal and tested in vitro even when captopril

 Our finding that captopril inhibits the development of experimental autoimmune myocarditis is supported by several studies showing the beneficial effects of captopril on human cardiomyopathies (12, 13), infection-induced experimental myocarditides (14–20), and experimental autoimmune disease models (48, 49). In addition, another ACE inhibitor, temocapril, reduces myosin-induced myocarditis in rats via redox regulation mechanisms involving thioredoxin, although the effect of this agent on the immune system is not known (50). Our data on the reduction of DTH responses by captopril are novel and are supportive of the drug's role in suppressing inflammatory processes (reviewed in Ref. 11). Captopril reduced both heart weight and heart weight to body weight ratio almost to normal, as is true of other disease models (14–19, 51). This reduction is due to a decreased inflammation, myocyte necrosis, and consequent reparative fibrosis (Table II). Our data also indicate that captopril completely prevents myocarditis in some mice and reduces disease severity in those mice that
did develop some disease (Table II). Although the reduction in disease severity in affected mice does not reach statistical significance, it may still be of clinical significance. We are currently addressing whether treatment of captopril at later times postdisease induction also reduces myocarditis, as is true of mice infected with coxsackievirus B3 (17, 18).

The precise mechanism by which captopril reduces autoimmune myocarditis remains to be determined. We initially hypothesized that captopril functions by inhibiting myosin-specific T cell responses. Although we found that captopril decreased myosin DTH in myosin-immunized mice (Fig. 3), we also found that captopril could work locally by reducing myosin DTH when injected into the DTH test site. In addition, the reduction in myosin DTH is not due to the cardioprotective role of captopril (less damage leading to decreased autoimmune response (21)), because captopril also reduced OVA DTH in OVA-immunized mice. These results suggest that captopril may suppress cell-mediated immunity against a self or foreign Ag irrespective of the presence of myocardial damage. We wondered whether the reduction in DTH induced by captopril was due to suppression of T cell function, as reported by other groups (42, 49). Instead, we found that captopril, whether administered in vitro or in vivo, had no effect on the proliferative capacity or cytokine secretion of splenocytes stimulated by Ag or by anti-CD3 (Fig. 5). Our results are in accordance with those showing that proinflammatory and anti-inflammatory cytokines secreted by human PBMCs are not affected by captopril administration (52). These results are also counter to those of other groups

FIGURE 5. In vitro captopril administration does not affect Ag-specific T cell proliferation or cytokine secretion by splenocytes of OVA-immunized mice. At 21 days postimmunization, splenocytes were prepared and cultured with 10 μM OVA, 10 μM myosin (Myosin) as a negative control, or anti-CD3 (anti-CD3) as a positive control, from four groups of mice: OVA immunized and captopril treated (OVA/CFA + Captopril), OVA immunized (OVA/CFA), saline immunized and captopril treated (PBS/CFA + Captopril), and saline immunized (PBS/CFA). Captopril was administered to mice from day of immunization to day of sacrifice. Splenocytes were pooled from at least five mice in each group and did not receive captopril in the culture medium. A, Proliferative responses were measured by [3H]Tdr incorporation and represented as the mean of triplicate wells. B, Culture supernatants were assayed for the presence of IL-2 or IFN-γ secreted by splenocytes cultured with 10 μM OVA or 10 μM myosin. Splenocytes were pooled from at least five mice. Symbols represent the mean of triplicate wells. Error bars represent SEM.

FIGURE 6. In vivo captopril administration does not affect Ag-specific T cell proliferation or cytokine secretion by splenocytes of OVA-immunized mice. At 21 days postimmunization, splenocytes were prepared and cultured with 10 μM OVA, 10 μM myosin (Myosin) as a negative control, or anti-CD3 (anti-CD3) as a positive control, from four groups of mice: OVA immunized and captopril treated (OVA/CFA + Captopril), OVA immunized (OVA/CFA), saline immunized and captopril treated (PBS/CFA + Captopril), and saline immunized (PBS/CFA). Captopril was administered to mice from day of immunization to day of sacrifice. Splenocytes were pooled from at least five mice in each group and did not receive captopril in the culture medium. A, Proliferative responses were measured by [3H]Tdr incorporation and represented as the mean of triplicate wells. B, Culture supernatants were assayed for the presence of IFN-γ and IL-2 and represented as the mean of triplicate wells. Error bars represent SEM.
reporting that captopril enhances T cell function in their model systems (53–56). The main difference between our results and those of the other groups is that we tested the effect of captopril on Ag-specific T cell proliferation and cytokine secretion, while other groups addressed this question in an Ag-nonspecific manner (e.g., by Con A, LPS, and PHA responses, among others). We do not yet know the mechanism by which captopril affects T cell responsiveness in vivo. Some groups have suggested that differences in these effects could be due to duration of captopril exposure (57), dosage (52–54, 58), in vitro vs in vivo administration (54), and plasma levels of PGs and bradykinin (56, 59), among other possibilities. We are currently addressing these hypotheses in our model system. If captopril does not directly affect T cell function, captopril may reduce inflammation and DTH by reducing recruitment of T cells to the site of antigenic stimulation or by altering the local inflammatory environment. These hypotheses are supported by our result that local injection of captopril into the DTH site reduced DTH. We are currently testing both hypotheses. Captopril has been shown to inhibit lymphocyte recruitment by decreasing chemotaxis in capillary endothelial cells (43) and neutrophils (44). The effects of captopril on chemokines have not been addressed, but there is mounting evidence that angiotensin II affects the activities of a number of chemokines, including RANTES (CCL-5), monocyte chemoattractant protein-1 (CCL-2), and macrophage-inflammatory protein 1-α (CCL-3) (reviewed in Ref. 11). Captopril also reduces local inflammatory processes necessary for the induction of DTH responses, including: deposition of extravasated fibrin (17); production of proinflammatory cytokines such as TNF-α (40, 41), IFN-γ (42), and IL-12 (42); and dendritic cell function (45). Taken together, these results show that captopril significantly reduces experimental autoimmune myocarditis. Additional mechanisms of action of captopril could involve suppression of angiotensin II levels, enhancement of bradykinin levels, or a pharmacologic effect of captopril’s thiol group, among other mechanisms. Antagonists of angiotensin II receptors reduced encephalomyocarditis virus-induced myocarditis (16, 51, 60). Increased bradykinin levels and activation of NO and PGs by ACE inhibitors have been implicated in providing cardiac protection via reduction of infarct size (61), reduction of hypertrophy (62), and reduction of collagen gene expression (63). The cardioprotective effect may also be due to up-regulation of bradykinin, leading to NO synthesis (63, 64), which is most likely an important molecule in autoimmune myocarditis (65). Finally, the thiol group of captopril is thought to ameliorate encephalomyocarditis virus-induced myocarditis by elimination of oxygen radicals (14).

In conclusion, captopril ameliorates autoimmune myocarditis, as shown by its reduction of cardiac hypertrophy, inflammation, necrosis, and fibrosis. Our data suggest that captopril reduces inflammation by decreasing cell-mediated immunity, without a direct effect on T cells, but not by affecting humoral immunity. These results have direct clinical import because patients suffering from autoimmune myocarditis would benefit from captopril. Future work from our laboratory will address the mechanisms of action of captopril in decreasing inflammation, so as to increase the specificity of treatment.

Acknowledgments

We thank Dr. William Ward for the kind gift of captopril, and Joann Taylor-Hinz for technical expertise in performing the ACE activity assay. We thank Dr. Alfred Rademaker (Center for Preventative Medicine, Northwestern University) for advice on statistical analysis, and Drs. Stephen Miller, William Karpus, and Carl Waltenbaugh for reagents and advice.

References


