IL-1-Driven Endogenous IL-10 Production Protects Against the Systemic and Local Acute Inflammatory Response Following Intestinal Reperfusion Injury

Danielle G. Souza, Rodrigo Guabiraba, Vanessa Pinho, Adrian Bristow, Stephen Poole and Mauro M. Teixeira

*J Immunol* 2003; 170:4759-4766; doi: 10.4049/jimmunol.170.9.4759

http://www.jimmunol.org/content/170/9/4759

**References** This article cites 43 articles, 11 of which you can access for free at: http://www.jimmunol.org/content/170/9/4759.full#ref-list-1

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

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

**Email Alerts** Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
IL-1-Driven Endogenous IL-10 Production Protects Against the Systemic and Local Acute Inflammatory Response Following Intestinal Reperfusion Injury

Danielle G. Souza,* Rodrigo Guabiraba,* Vanessa Pinho,* Adrian Bristow,† Stephen Poole,† and Mauro M. Teixeira2*

TNF-α release and action are central in the pathogenesis of the local and systemic inflammatory responses that occur after intestinal reperfusion. In this study we examined whether IL-1 participated in the cascade of events leading to TNF-α production and TNF-α-mediated injury following reperfusion of the ischemic superior mesenteric artery in rats. Blockade of the action of IL-1 by the use of anti-IL-1 antiserum or administration of IL-1R antagonist (IL-1ra), a natural antagonist of IL-1Rs, resulted in marked enhancement of reperfusion-associated tissue injury, TNF-α expression, and lethality. In contrast, there was marked decrease in IL-10 production. Facilitation of IL-1 action by administration of anti-IL-1ra, which antagonizes endogenous IL-1ra, or exogenous administration of rIL-1β suppressed reperfusion-induced tissue pathology, TNF-α production, and lethality, but increased IL-10 production. Exogenous administration of IL-10 was effective in preventing the increase in tissue or plasma levels of TNF-α, the exacerbated tissue injury, and lethality. An opposite effect was observed after treatment with anti-IL-10, demonstrating a role for endogenous production of IL-10 in modulating exacerbated reperfusion-associated tissue pathology and lethality. Finally, pretreatment with anti-IL-10 reversed the protective effect of IL-1β on reperfusion-associated lethality. Thus, IL-1 plays a major role in driving endogenous IL-10 production and protects against the TNF-α-dependent systemic and local acute inflammatory response following intestinal reperfusion injury. The Journal of Immunology, 2003, 170: 4759–4766.

*Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil; †Division of Endocrinology, National Institute for Biological Standards and Control, South Mimms, Potters Bar, United Kingdom

Received for publication September 11, 2002. Accepted for publication March 3, 2003.

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

1 This work was supported by Fundação Amparo a Pesquisas do Estado de Minas Gerais and Conselho Nacional de Desenvolvimento Científico e Tecnológico.

2 Address correspondence and reprint requests to Dr. Mauro Martins Teixeira, Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Avenue Antonio Carlos, 6627 Pampulha, 31270-901 Belo Horizonte MG, Brazil. E-mail address: mmtex@icb.ufmg.br

Copyright © 2003 by The American Association of Immunologists, Inc.

0022-1767/03/$02.00

Abbreviations used in this paper: TNFR1, TNF-α receptor 1; IL-1ra, IL-1R antagonist; SMA, superior mesenteric artery.
Materials and Methods

Animals

Male Wistar rats (200–220 g) obtained from the bioscience unit of our institution were housed under standard conditions and had free access to commercial chow and water. All procedures described here had prior approval from the institutional animal ethics committee.

Ischemia and reperfusion injury

Rats were anesthetized with urethane (140 mg/kg i.p.), and laparotomy was performed. The superior mesenteric artery (SMA) was isolated, and ischemia was induced by totally occluding the SMA for 120 min. The abdominal incision was occluded with gauze moistened with warm saline. After ischemia, reperfusion was initiated by removal of the occlusion and was maintained for 120 min (5). The temperature of the animals was maintained with a warm blanket, and no fluid was given throughout the observation period. sham-operated animals or animals only made ischemic were used as controls for the reperfusion-induced injury. Preliminary experiments showed that there was variation in the degree of inflammatory damage between animals that died just after reperfusion and those that survived for 120 min of reperfusion (data not shown). For this reason and to maintain homogeneity within a particular group, inflammatory parameters were assessed only in animals that were alive after 120 min of reperfusion.

Anti-IL-1 (0.5 ml of hyperimmune serum/animal), anti-IL-10 (0.5 ml/animal), anti-IL-1ra (0.5 ml/animal), recombinant human IL-1ra (2 mg/kg), recombinant rat IL-1β (1 µg/animal), or recombinant rat IL-10 (1 µg/animal) was administered s.c. 60 min before reperfusion, thus 60 min after the onset of ischemia. The anti-IL-1 hyperimmune serum was a 50/50 mixture of anti-IL-1β and anti-IL-1α. In a separate series of experiments IL-1α (2 mg/kg) was given i.v. 10 min before reperfusion. In addition, in another series of experiments evaluating the ability of anti-IL-10 to reverse the effects of IL-1β, anti-IL-10 (0.5 ml/animal) was given s.c. at a distinct site from and 10 min before the administration of IL-1β (1 µg/animal). Polyclonal Abs were raised in sheep, and recombinant proteins were cloned and prepared as previously described (19–21). The dose of IL-1α chosen for our experiments has been shown to reduce limb ischemia and reperfusion injury in rats (18).

Evaluation of changes in vascular permeability and neutrophil accumulation

The extravasation of Evans Blue dye into the intestine, mesentery, and left lung tissue was used as an index of increased vascular permeability, as previously described (5). Results are presented as the amount of Evans Blue per microgram per 100 mg of tissue. The extent of neutrophil accumulation in the intestine, mesentery, and right lung tissue was measured by assaying myeloperoxidase activity, as previously described (5). Results are expressed as the total number of neutrophils by comparing the OD of tissue supernatant with the OD of rat peritoneal neutrophils processed in the same way as tissue samples.

Determination of the concentrations of circulating leukocytes

The total numbers of circulating leukocytes and neutrophils were evaluated in blood samples obtained via a cannula in the femoral artery. Samples were collected before ischemia (time zero), 120 min after ischemia, and 30 and 120 min after reperfusion. The number of total circulating leukocytes was determined by counting leukocytes in a modified Neubauer chamber after staining with May–Grunwald–Giemsa.

Measurement of hemoglobin concentrations

Determination of the concentration of hemoglobin in intestinal tissue using Drabkin’s color reagent was used as an index of tissue hemorrhage, as previously described (5).

Measurement of cytokine concentrations in serum, intestine, and lungs

TNF-α, IL-1β, IL-6, and IL-10 concentrations were measured in the serum and intestine of animals using ELISA techniques previously described (19–24). Briefly, serum was obtained from coagulated blood (15 min at 37°C, then 30 min at 4°C) and stored at −20°C until further analysis. Serum samples were analyzed at a 1/1 dilution in PBS. One hundred milligrams of duodenum or lung of sham-operated and reperfused animals were homogenized in 1 ml of PBS (0.4 M NaCl and 10 mM NaPO₄) containing anti-proteases (0.1 mM PMSF, 0.1 mM benzethonium chloride, 10 nM EDTA, and 20 KI of aprotinin A) and 0.05% Tween 20. The samples were then centrifuged for 10 min at 3000 × g, and the supernatant was immediately used for ELISA assays at a 1/5 dilution in PBS. ELISA plates (Nunc MaxiSorb) were coated with sheep anti-rat TNF-α/IL-1β/IL-6 or IL-10 polyclonal Abs (1–2 µg/ml) overnight. The plates were washed three times and then blocked with 1% BSA. After a further wash, plates were incubated with samples or recombinant rat cytokine and incubated overnight. The biotinylated polyclonal Abs were used at a 1/1000 to 1/2000 dilution, and the assays had a sensitivity of 16 pg/ml.

Statistical analysis

Results are shown as the mean ± SEM. The percent inhibition was calculated by subtracting the background concentrations of Evans Blue extravasation or myeloperoxidase (obtained in sham-operated animals) from control and treated animals. Differences were compared using ANOVA, followed by Student-Newman-Keuls post-hoc analysis. Results with a statistical difference of $p < 0.05$ were considered significant. For survival curves, differences between groups at different time points were compared using Fisher’s exact test and were considered significant at $p < 0.05$.

Results

For the experiments described below, control animals received saline or nonimmune sheep serum. As nonimmune serum had no effect on injuries following reperfusion of the ischemic SMA, results in nonimmune serum- and saline-treated animals were pooled for presentation (for example, myeloperoxidase detection in lung: vehicle-treated, 5.6 ± 0.28 neutrophils × 10⁶/lung; nonimmune serum-treated, 6.32 ± 0.67; TNF-α concentration in serum: vehicle-treated, 3617.56 ± 230.8 pg/ml; nonimmune serum-treated, 2766.5 ± 190.0).

Strategies that prevent IL-1β action enhance reperfusion injury

Initial experiments examined the ability of strategies known to suppress the action of IL-1β: pretreatment with anti-IL-1 Ab (0.5 ml s.c.) or prevention of IL-1β action by administration of IL-1ra (2 mg/kg s.c.). To our surprise, treatment with both anti-IL-1 and IL-1ra significantly increased the number of infiltrating neutrophils in the intestine and lungs after ischemia and reperfusion injury (Fig. 1, A and C). The increase in neutrophil numbers in tissue was accompanied by a significant increase in vascular permeability in the intestine and lungs of reperfused animals (Fig. 1, B and D). The extravasation of hemoglobin, an index of tissue hemorrhage, was similar in animals treated with vehicle, anti-IL-1, or IL-1ra (Fig. 1E). In addition, either of these strategies failed to significantly affect the rapid neutropenia that followed the reperfusion of the ischemic superior mesenteric artery (data not shown). Virtually identical results were obtained when IL-1ra was administered i.v. 10 min before reperfusion (data not shown).

Reperfusion injury is accompanied by a marked local (intestine, remote (lung), and systemic increase in the concentration of proinflammatory cytokines, including TNF-α, and anti-inflammatory cytokines, such as IL-10. Consistent with the enhanced tissue injury observed above, treatment with anti-IL-1 or IL-1ra induced a further increase in the concentration of TNF-α in the lungs (Table I) and serum (Fig. 2) of reperfused animals. The increase in TNF-α concentrations in the intestine after reperfusion was similar in treated and untreated animals (Table I). Overall, concentrations of IL-6 in tissue were similar in control and anti-IL-1-treated animals, whereas treatment with IL-1ra enhanced the increase in IL-6 in tissues (Table I). Either treatment failed to affect the increase in serum concentrations of IL-6 (data not shown). In contrast to their ability to enhance TNF-α secretion, treatment with anti-IL-1 or IL-1ra induced a marked decrease in the concentration of IL-10 in the serum (Fig. 2) and tissue (Table I) of reperfused animals.

Intestinal ischemia and reperfusion injury is accompanied by TNF-α-dependent lethality that generally approaches 50% after 120 min of reperfusion (5, 6). In accordance with their ability to...
enhance the concentrations of TNF-α, treatment with anti-IL-1 or IL-1ra also enhanced the lethality associated with intestinal reperfusion (Fig. 3).

Administration of IL-1β or blockade of IL-1ra protect from reperfusion injury

As strategies that blocked IL-1β action tended to enhance the injuries and lethality associated with intestinal ischemia and reperfusion, we tested the effects of strategies that mimicked or facilitated the effects of IL-1β. To this end, animals were pretreated systemically with recombinant rat IL-1β or were given an antagonist of IL-1ra, which neutralizes IL-1ra, an endogenous inhibitor of IL-1Rs. Both of the latter strategies were given shortly before reperfusion and markedly prevented the influx of neutrophils in lungs and intestine after intestinal ischemia and reperfusion injury (Fig. 1). Similarly, pretreatment with IL-1β or anti-IL-1ra greatly diminished the increase in vascular permeability and hemorrhage (Fig. 1). In contrast, these treatments failed to significantly affect the neutropenia that followed reperfusion (data not shown).

The ability of IL-1β and anti-IL-1ra to inhibit local and remote inflammation was mimicked by the ability of these treatments to suppress reperfusion-induced production of TNF-α and IL-6 (Fig. 2 and Table I). Not only did IL-1β and anti-IL-1ra decrease the production of pro-inflammatory cytokines, but these treatments also enhanced the release of IL-10 in serum (Fig. 2) and tissues (Table I) after intestinal ischemia and reperfusion injury.

FIGURE 1. IL-1 and reperfusion-associated increase in neutrophil influx, vascular permeability, and hemorrhage. Strategies that prevent (anti-IL-1 or IL-1ra) or enhance/mimic (exogenous IL-1β or anti-IL-1ra) the action of IL-1 were evaluated for the influx of neutrophils (A and B) and the increase in vascular permeability (C and D) into the intestine (A and C) and lungs (B and D) following ischemia and reperfusion (I/R) of the superior mesenteric artery in rats. The increase in hemorrhage into the intestine (E) was also evaluated. Neutrophil accumulation was assessed by measuring the tissue content of myeloperoxidase, changes in vascular permeability by the extravasation of Evans Blue dye, and hemorrhage by the intestinal levels of hemoglobin. Abs, recombinant proteins, or vehicle were administered before reperfusion as described in Materials and Methods. Results are shown as the number of neutrophils or micrograms of Evans Blue dye per 100 mg of tissue and are the mean ± SEM of at least six animals per group. *, p < 0.05 compared with sham-operated animals; #, p < 0.05 compared with vehicle-treated severe ischemic/reperfused animals.
In addition to inhibiting tissue inflammation and the increase in tissue and systemic concentrations of proinflammatory cytokines, treatment with IL-1β or anti-IL-1ra was accompanied by prevention of reperfusion-associated lethality (Fig. 3).

### Table 1. IL-1β, IL-10, and reperfusion-associated increase in tissue concentrations of cytokines

<table>
<thead>
<tr>
<th>Cytokines (pg/100 mg of tissue)</th>
<th>TNF-α</th>
<th>IL-6</th>
<th>IL-10</th>
<th>IL-1β</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intestine</td>
<td>Lung</td>
<td>Intestine</td>
<td>Lung</td>
</tr>
<tr>
<td>Sham</td>
<td>42 ± 3</td>
<td>21 ± 2</td>
<td>5 ± 0.4</td>
<td>Not detectable</td>
</tr>
<tr>
<td>Severe I/R Strategies that prevent IL-1 action</td>
<td>689 ± 70³</td>
<td>587 ± 52³</td>
<td>136 ± 10³</td>
<td>868 ± 94³</td>
</tr>
<tr>
<td>Anti-IL-1</td>
<td>553 ± 22</td>
<td>1985 ± 191</td>
<td>172 ± 11³</td>
<td>886 ± 48</td>
</tr>
<tr>
<td>IL-1ra</td>
<td>780 ± 66</td>
<td>1957 ± 168</td>
<td>344 ± 38³</td>
<td>1912 ± 102³</td>
</tr>
<tr>
<td>Strategies that mimic/ enhance IL-1 action</td>
<td>61 ± 7³</td>
<td>21 ± 2³</td>
<td>86 ± 7³</td>
<td>196 ± 19³</td>
</tr>
<tr>
<td>IL-1β</td>
<td>37 ± 2³</td>
<td>20 ± 2³</td>
<td>7 ± 1³</td>
<td>Not detectable</td>
</tr>
<tr>
<td>Anti-IL-1ra</td>
<td>46 ± 3³</td>
<td>21 ± 2³</td>
<td>39 ± 3³</td>
<td>Not detectable</td>
</tr>
<tr>
<td>IL-10-based strategies</td>
<td>722 ± 43</td>
<td>989 ± 88³</td>
<td>193 ± 10³</td>
<td>1017 ± 54³</td>
</tr>
<tr>
<td><strong>Anti-IL-10</strong></td>
<td>284 ± 22</td>
<td>2106 ± 143³</td>
<td>71 ± 7³</td>
<td>464 ± 37³</td>
</tr>
</tbody>
</table>

* Strategies that prevent (anti-IL-1 or IL-1ra) or enhance/mimic (exogenous IL-1β or anti-IL-1ra) the action of IL-1β and strategies that prevent (anti-IL-10) or mimic (exogenous IL-10) the action of IL-10 were given prior to reperfusion, as shown in Materials and Methods. Results are shown as the mean ± SEM of at least six animals in each group. p ≤ 0.05 compared to sham-operated animals. `p ≤ 0.05 compared to vehicle-treated severe I/R animals.

Endogenous or exogenous IL-10 are protective during reperfusion injury

The contrasting effects of treatments that modify/mimic IL-1β action on the serum concentrations of TNF-α and IL-10 are clearly shown in Fig. 2. We have previously demonstrated an important role for TNF-α in our model (6). As endogenous production or exogenous administration of IL-10 may modulate the acute inflammatory response that follows ischemia and reperfusion injury to some vascular beds (reviewed in Ref. 25), it was of interest to investigate the role of this cytokine in our system.

Fig. 4 shows the effects of exogenous IL-10 administration and anti-IL-10 treatment on the injuries to the intestine that occur after ischemia and reperfusion. Thus, whereas IL-10 treatment prevented influx of neutrophils, increase in vascular permeability, and hemorrhage after reperfusion injury, treatment with anti-IL-10 tended to enhance intestinal injury (Fig. 4). Virtually identical results were seen when reperfusion-induced inflammation was assessed in the lungs, i.e., prevention of injury by IL-10 and enhancement by anti-IL-10 (data not shown).

![Figure 2](http://example.com/figure2.png)

**Figure 2.** IL-1 and reperfusion-associated increase in serum concentrations of TNF-α and IL-10. Strategies that prevent (anti-IL-1 or IL-1ra) or enhance/mimic (exogenous IL-1β or anti-IL-1ra) the action of IL-1 were evaluated for the increase in concentrations of TNF-α in serum following ischemia and reperfusion (I/R) of the SMA in rats. TNF-α and IL-10 were measured using specific ELISAs. Abs, recombinant proteins, or vehicle were administered before reperfusion as described in Materials and Methods. Results are shown as picograms of the cytokine per 100 mg of tissue and are the mean ± SEM of at least six animals in each group. * p < 0.05 compared with sham-operated animals; #, p < 0.05 compared with vehicle-treated severe ischemic/reperfused animals.

![Figure 3](http://example.com/figure3.png)

**Figure 3.** IL-1 and reperfusion-associated lethality. Strategies that prevent (anti-IL-1 or IL-1ra) or enhance/mimic (exogenous IL-1β or anti-IL-1ra) the action of IL-1 were evaluated for the lethality that follows ischemia and reperfusion of the superior mesenteric artery in rats. Abs, recombinant proteins, or vehicle were administered before reperfusion as described in Materials and Methods. Survival was monitored as indicated, and survivors were sacrificed after 120 min. There were 10 animals per experimental group, except in the vehicle (n = 15) and IL-1ra (n = 14) groups.
The ability of IL-10 to prevent tissue injury was associated with a marked decrease in tissue (Table I) and serum (Fig. 5A) levels of TNF-α/H9251 and prevention of lethality (Fig. 5B). In contrast, anti-IL-10 treatment enhanced tissue (Table I) and serum (Fig. 5A) TNF-α/H9251 levels as well as lethality (Fig. 5B). Similarly, pretreatment with IL-10 prevented, whereas anti-IL-10 markedly enhanced, the increase in tissue (Table I) and serum (data not shown) concentrations of IL-6. On the contrary, the increase in the tissue concentration of IL-1/H9252 was enhanced by IL-10 and suppressed by anti-IL-10 pretreatment (Table I). A similar situation was observed in serum (sham, 67 ± 7 pg IL-1/H9252/ml of serum; ischemia and reperfusion, 229 ± 33 pg/ml; ischemia, reperfusion, and IL-10 treatment, 970 ± 95 pg/ml; ischemia, reperfusion, and anti-IL-10 treatment, 89 ± 7; n = 6, p < 0.01). It is clear from the results presented above that endogenously produced or exogenously added IL-10 have a protective effect against reperfusion-associated injury and lethality in our model.

**IL-10 mediates the protective effects of IL-1β on reperfusion injury**

As the protective effect of IL-1β administration on ischemia and reperfusion injury was associated with enhancement of IL-10 production, and IL-10 appeared to play an important protective role in our system, we then investigated whether anti-IL-10 would block the protective effect of IL-1β on reperfusion-associated lethality. Similar to the results presented in Fig. 3, pretreatment with IL-1β totally prevented the lethality associated with ischemia and reperfusion injury (Fig. 6). However, pretreatment with anti-IL-10 reversed the protective effect of IL-1β on reperfusion-associated lethality (Fig. 6).

**Discussion**

The restoration of blood flow of an ischemic vascular bed, i.e., reperfusion, is a major therapeutic objective following ischemia of an organ or tissue (1–4). However, the reperfusion of an ischemic bed may lead to inflammation locally and systemically, limiting...
the potential benefits of blood flow restoration. Thus, strategies that limit the injury caused by the reperfusion process may be a useful adjunct in the treatment of acute ischemic disorders in various organs (1–4).

In a model of reperfusion injury following a prolonged period of ischemia (120 min) of the superior mesenteric artery, there is a massive local, remote, and systemic inflammatory response and significant lethality (5–8). Our previous work has shown an important role for TNF-α in reperfusion-induced tissue injury and lethality (5–8). This is in marked agreement with other studies demonstrating a central role for TNF-α during reperfusion-associated injury in several vascular beds (1, 2, 10, 11, 18). As IL-1β may be involved in the induction of TNF-α during acute and chronic inflammatory conditions, we evaluated the ability of IL-1β to modulate TNF-α production, tissue injury, and lethality in our model of severe injury following ischemia and reperfusion of the superior mesenteric artery in rats.

Two strategies were used to block the action of IL-1β neutralization of the protein with anti-IL-1 antisera and administration of IL-1ra, a natural antagonist of IL-1R. Our results clearly show that the use of both strategies was associated with an overall enhancement of tissue injury, proinflammatory cytokine expression, and lethality. These were surprising results, as previous studies have suggested a role for IL-1β during models of less severe ischemia and reperfusion injury (10–12, 14–18). Indeed, IL-1β has been implicated in the expression of cell adhesion molecules and neutrophil influx following ischemia and reperfusion injury (14–18). However, other models have also failed to show a protective effect of IL-1β inhibition during ischemia and reperfusion injury (26–28). One possible difference between these studies is the severity of the local and systemic inflammatory responses that occur after ischemia and reperfusion. Under the conditions described in our experiments, local and systemic inflammatory responses are rapid, massive, and accompanied by significant lethality. Interestingly, neutralization and/or antagonism of IL-1β were associated with a marked prevention of the increase in IL-10 that occurs following reperfusion injury. The latter results suggest that IL-1β is a major inducer of IL-10 during severe intestinal ischemia and reperfusion injury. Moreover, we have previously shown that TNF-α also contributed, but to a lesser extent, to IL-10 production during intestinal ischemia and reperfusion (6) (see Fig. 7).

As neutralization or blockade of IL-1β action was accompanied by enhancement of injury, it was of interest to examine whether the opposite effect would be observed if IL-1β action was enhanced. Two strategies were used to this effect: administration of anti-IL-1Ra, which antagonizes endogenous IL-1ra, and exogenous administration of rIL-1β. Both strategies suppressed tissue pathology and increased the proinflammatory cytokines and lethality that followed reperfusion of the ischemic superior mesenteric artery. Of note, there was a marked inhibition of the elevation of TNF-α levels in tissue and serum. On the contrary, blockade of IL-1β was accompanied by enhancement of tissue and serum concentrations of IL-10. Several studies have previously demonstrated the protective effects of exogenous administration of IL-1β during ischemia and reperfusion injury (29–31). However, in these studies, IL-1β was given at least 24 h before ischemia and the effects associated with tissue increase in the levels of enzymes with antioxidant activity, including superoxide dismutase, catalase, and glucose-6-phosphate dehydrogenase (29–31). This IL-1β-induced preconditioning of tissue (32) is unlikely to occur in our system, as the cytokine was given after ischemia had started and immediately before reperfusion.

One possibility for the inhibitory role of IL-1β in our system was raised by the demonstration that endogenous or exogenous IL-1β modulated the production of IL-10. Several studies have demonstrated that IL-10 modulates proinflammatory cytokine production and tissue injury following ischemia and reperfusion injury (25, 33–35). For example, studies in IL-10-deficient mice suggested that endogenous IL-10 had an anti-inflammatory role during reperfusion injury, possibly by regulating an early stress genetic

---

**FIGURE 6.** The protection of reperfusion-induced lethality afforded by IL-1β is IL-10 dependent. Anti-IL-10 or nonimmune rabbit serum was administered before IL-1β and before reperfusion of the ischemic SMA, as described in Materials and Methods. Survival was monitored as indicated, and survivors were sacrificed after 120 min. There were nine animals in each experimental group.

---

**FIGURE 7.** Schematic representation of the roles of TNF-α, IL-1β, and IL-10 during severe intestinal ischemia and reperfusion injury. See text for further details.
response (c-Jun kinase activation), neutrophil influx, and cytokine production (34). Exogenous administration of IL-10 reduced the systemic inflammatory response in a murine model of intestinal reperfusion injury, an effect associated with inhibition of cytokine production and neutrophil accumulation (35). In our model, exogenous administration of IL-10 was effective in preventing the increase in tissue or plasma levels of TNF-α, the exacerbated tissue injury, and lethality. Moreover, treatment with anti-IL-10 was associated with increased TNF-α concentration, tissue injury, and lethality, demonstrating a role for endogenous production of IL-10 in modulating exacerbated tissue pathology and lethality. As IL-10 prevents tissue injury and lethality, and IL-1β appeared to play an important role in controlling IL-10 production, we evaluated whether this production of IL-10 was relevant for the actions of IL-1β. Our results showed that pretreatment with anti-IL-10 reversed the protective effects of IL-1β on reperfusion-associated lethality. Thus, during severe intestinal reperfusion injury, IL-1β production is a major driving force for the production of IL-10. The IL-10 produced ultimately mediates the suppressive effects of IL-1β in the model (Fig. 7).

Our previous studies have suggested that neutrophils were necessary for the production of tissue TNF-α (5–9). Thus, when the tissue influx of neutrophil was prevented (e.g., anti-CD18 and platelet-activating factor receptor antagonists), tissue production of TNF-α was also prevented. In the present series of experiments whenever there was inhibition of neutrophil influx (IL-1, anti-IL-1ra, IL-10), there was also inhibition of tissue TNF-α production. In contrast, strategies associated with enhanced neutrophil influx (anti-IL-1, IL-1ra, anti-IL-10) were also associated with an increase in TNF-α production in the lungs. In the intestine, the increase in neutrophil influx induced by the latter strategies was not accompanied by any major increase in TNF-α production (or increase in hemorrhage; see Fig. 1E). However, neither hemorrhage nor TNF-α production occurred in the absence of neutrophils (5–9). Thus, it appears that in the lungs the enhanced accumulation of neutrophils is capable of inducing greater production of cytokines (such as TNF-α) and possibly inflicting further damage. In the intestine the enhanced accumulation of neutrophils is not sufficient to enhance cytokine production or tissue hemorrhage. The reasons underlying the different responses of these organs to the increased neutrophil concentration are not known, but could be due to the different ability of resident cells to interact with the migrating neutrophils. Together these studies suggest an important role for the tissue influx of neutrophils in driving the tissue production of TNF-α.

A final interesting observation was the ability of anti-IL-10 to suppress and of exogenous IL-10 to enhance the tissue and serum concentrations of IL-1β. One possibility to explain these intriguing results is that TNF-α may be inducing an intermediate molecule that controls IL-1β production (Fig. 7). The latter hypothesis is consistent with our previous studies demonstrating that inhibition of TNF-α is accompanied by marked inhibition of lethality, but enhancement of reperfusion-induced production of IL-1β (6). The identification of this putative TNF-α-induced factor capable of preventing the increase in IL-1β clearly deserves further investigation. One possible candidate is TGF-β, an NF-κB-inducible cytokine known to possess anti-inflammatory effects and to be expressed during ischemia and reperfusion injury (36–38). In conclusion, the results presented herein show that strategies that antagonized IL-1β enhanced, whereas strategies that favored IL-1 action prevented, reperfusion-induced tissue injury and lethality. The effects of IL-1 appeared to work via modulation of IL-10 production and consequent modulation of TNF-α-dependent tissue injury and lethality. A situation is envisaged in which there is generation of oxidative stress and activation of lipid mediators and protease cascades (both bradykinin and complement) shortly after reperfusion of the ischemic superior mesenteric artery (Fig. 7). The latter elements of the reperfusion-associated inflammatory response may be sufficient to activate mitogen-activated protein kinases and NF-κB (38–43). Activation of these transcription factors appears to be sufficient to induce the rapid (within 2 h, the time frame of the experiment) production of both IL-1β and TNF-α. In our model TNF-α is central to the pathogenesis of reperfusion-associated injury and lethality (6, 8). However, both IL-1β and TNF-α appear to trigger anti-inflammatory cascades; the production of IL-10 is mainly under the control of IL-1β, whereas TNF-α appears to activate IL-10 and a distinct, yet unrecognized, molecule. The fast production of anti-inflammatory molecules is capable of preventing the exacerbated pathology triggered by severe ischemia and reperfusion injury. The present study highlights the intricate balance and cross-talk between pro- and anti-inflammatory cytokines during a systemic inflammatory response.

Acknowledgments

We are grateful to Dr. Fernando Cunha for helpful criticism.

References
