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Interleukin 1 receptor blockade attenuates the host inflammatory response

(anorexia/cachexia/cytokines/amyloid P)

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ABSTRACT **Cytokines, including interleukin 1 (IL-1), tumor necrosis factor α, and interleukin 6, are often produced in response to tissue injury and contribute to several host responses such as weight loss, anorexia, and acute-phase protein synthesis. However, the role of IL-1 in specific tissue responses is unclear. To test our hypothesis that specific *in vivo* blockade of IL-1's action might inhibit the catabolic host changes associated with inflammation, mice were passively immunized with a monoclonal antibody directed against the murine IL-1 receptor prior to initiation of a turpentine-induced sterile abscess. This antibody prevents IL-1-mediated proliferation of murine thymocytes *in vitro* by inhibiting IL-1α and IL-1β by way of competition for a common receptor. Weight loss following turpentine challenge was prevented by daily injections of anti-IL-1 receptor monoclonal IgG. Body composition analysis confirmed that lean tissue and fat were preserved by passive immunization. Furthermore, pretreatment with an anti-IL-1 receptor monoclonal antibody significantly attenuated the plasma amyloid P and interleukin 6 responses but did not affect the decline in plasma albumin or the increase in circulating corticosterone. Passive immunization of similar mice with polyclonal antisera against another cytokine, tumor necrosis factor α, failed to prevent either the weight loss or hepatic acute-phase protein changes observed in this inflammatory model. These findings suggest that IL-1 orchestrates weight loss and body compositional changes during inflammation and contributes to the induction of interleukin 6 and acute-phase protein synthesis.**

Several responses that characteristically result from tissue injury and inflammation include weight loss, anorexia, anemia, leukocytosis, and acute-phase protein synthesis (1). Although the consequences of injury can result in increased morbidity (2–4), the mechanisms underlying these changes in host physiology remain poorly defined. Several cytokines, including interleukin 1 (IL-1), tumor necrosis factor α (TNFα), and interleukin 6 (IL-6), are mediators of the host response to inflammation (5–10). Increased synthesis and release of these cytokines have been reported in patients or experimental animals during injury or inflammatory disease (11–17). IL-1 is one of several early mediators that initiates the tissue inflammatory response and induces the synthesis of several other inflammatory mediators (18–22). Consequently, we postulated that selective *in vivo* inhibition of IL-1 might broadly diminish aspects of the host inflammatory response.

The IL-1 receptor present on the murine T-cell clone EL-4 6.1 C10 has recently been isolated, purified, and cloned (23–25). This 80-kDa glycoprotein has also been detected on epithelial cells, hepatocytes, fibroblasts, and keratinocytes. The development of a specific monoclonal antibody against the murine T-cell/fibroblast type IL-1 receptor (anti-IL-1R mAb) has permitted further exploration of the role of IL-1 *in vivo*. This monoclonal antibody blocks D.10 T-cell and murine thymocyte proliferation in response to recombinant murine IL-1 *in vitro* (26). In this report, we examined whether passive immunization with anti-IL-1R mAb could prevent the catabolic host changes associated with a turpentine-induced sterile abscess. An advantage of this anti-IL-1R mAb is that selective blockade of the IL-1 receptor will simultaneously neutralize IL-1α and IL-1β biological activity.

MATERIALS AND METHODS

Antibody Preparations. Hybridoma cells secreting an anti-IL-1R mAb (35F5) were produced by fusing NSO cells with splenocytes recovered from a rat (no. 85-89) previously immunized with highly purified murine T-cell/fibroblast type IL-1 receptor, as described elsewhere (26). Antibodies were purified from ascites fluid of pristane-treated (2,6,10,14-tetramethylpentadecane; Aldrich) nude mice (*nu/nu*) by affinity chromatography on protein G-agarose (Genex). In preliminary studies, the administration of 200 μg of mAb administered to C57BL/6 mice 6 hr prior to challenge with 100 ng of murine IL-1α prevented the leukocytosis and elevation of acute-phase proteins that develop following IL-1α administration (27).

Polyclonal rabbit anti-murine TNFα antiserum was obtained from New Zealand White rabbits (Hazleton Research Products, Denver, PA) repeatedly immunized with murine TNFα (10–25 μg) that had been purified to homogeneity from endotoxin-stimulated RAW 264.7 murine macrophages. When 200 μl (≈2 mg of IgG) was administered i.p. to C57BL/6 mice 4 hr prior to a lethal endotoxin challenge, this antiserum conferred survival and blocked the appearance of TNFα in plasma (ref. 28 and unpublished observations). Nonimmune rabbit serum was obtained from the same rabbit or from additional rabbits prior to immunization.

Experimental Animals. Female C57BL/6 mice (Charles River Breeding Laboratories) were employed in all studies. Mice were allowed to equilibrate for at least 5 days in plastic shoe boxes with pine shavings (four animals per cage), and body weight and food intake were monitored daily to assess the general well-being of the animals. The protocol was approved by the Institutional Animal Care and Use Committee of Cornell University Medical College.

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Abbreviations: IL-1, interleukin 1; IL-6, interleukin 6; TNFα, tumor necrosis factor α; anti-IL-1R mAb, anti-interleukin 1 receptor monoclonal antibody.

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In studies designed to evaluate the cytokine, acute-phase protein, and corticosterone responses to IL-1 receptor blockade during experimental inflammation, female C57BL/6 mice were passively immunized i.p. with 200 μl of 0.9% NaCl containing either 200 μg of affinity-purified monoclonal rat anti-murine IL-1 receptor IgG (anti-IL-1R mAb) or an equal amount of nonimmune rat IgG (Sigma). Six hours later, mice were injected s.c. with 100 μl of steam-distilled turpentine into the right hindlimb. At 0, 2, 4, 8, 12, 24, 48, and 72 hr following turpentine administration, mice were sacrificed by cervical dislocation, and venous blood obtained by cardiac puncture was collected via heparinized syringe. In addition, for corticosterone analysis, blood samples were collected from undisturbed mice within 2 min of initial contact to minimize spurious corticosterone release during handling.

In additional studies, mice were passively immunized i.p. with 200 μl of polyclonal rabbit anti-murine TNFα antiserum 6 hr prior to induction of a turpentine abscess. Animals were sacrificed 0, 2, 4, 8, 12, 24, 48, and 72 hr later, and results were compared to similar mice passively immunized with either nonimmune rabbit serum or 200 μg of rat anti-IL-1R mAb.

In studies that evaluated the effect of specific IL-1 receptor inhibition on weight loss, anorexia, and depletion of carcass fat and protein during experimental inflammation, mice were passively immunized i.p. with 200 μl of 0.9% NaCl containing either 200 μg of anti-IL-1R mAb or an equal amount of nonimmune rat IgG as above. Six hours later, 100 μl of steam-distilled turpentine or 0.9% NaCl was injected s.c., as above. Food intake and body weight were measured every 24 hr for the next 6 days ($n = 20$ mice per treatment). In addition, mice were given daily passive immunizations of either 200 μg of i.p. anti-IL-1R mAb or an equivalent amount of nonimmune rat IgG. Carcass compositional analysis was performed on a subset of animals from another study that were sacrificed at 72 hr following induction of the turpentine abscess, when weight loss was maximal in control animals that were challenged with turpentine.

Analytical Methods. The quantity of neutralizing monoclonal antibody in the plasma of mice that received anti-IL-1R mAb was determined by binding assays to plasma membranes of the murine T-cell clone, EL-4. Briefly, mouse serum (1:50–1:500 dilutions) or standard concentrations of anti-IL-1R mAb (0.016–320 ng/ml) were preincubated with membranes of EL-4 cells for 1 hr at 37°C. ^{125}I-labeled mouse IL-1α (^{125}I-IL-1α; 15–20 fmol) was added and the incubation was continued for 1 hr at 37°C. The assay mix was harvested directly onto Millipore GVWP filters to separate free from receptor-bound ^{125}I-IL-1α. The filters were subsequently washed three times with 3 ml of phosphate-buffered saline containing bovine serum albumin (1 mg/ml; Sigma). Total specific binding was determined in the absence of anti-IL-1R mAb. Nonspecific binding of mouse ^{125}I-IL-1α was determined in the presence of 50 nM unlabeled mouse IL-1α. The data were plotted as the percent of specific binding in the presence of standard concentrations of antibody. Anti-IL-1R mAb levels were determined by interpolation.

Circulating IL-1 activity was determined by a competitive receptor-ligand binding assay as described above for the detection of IL-1 neutralizing activity, with the exception that recombinant murine IL-1α was used to generate a standard curve (29). Samples were diluted 1:5 prior to analysis, and the sensitivity of the assay was ≈1.8 ng/ml of plasma.

TNFα bioactivity was assessed using the WEHI 164 clone 13 fibroblast cytotoxicity bioassay (30). Plasma samples were diluted 1:50 and added to $\approx 5 \times 10^4$ exponentially growing WEHI 164 clone 13 fibroblasts in 96-well flat-bottomed microtiter plates containing RPMI 1640 medium (Mediatech, Washington, DC), heat-inactivated fetal calf serum (10%; Hyclone), and actinomycin D (1 μg/ml; Dako, Parsippany, NJ). Following incubation for 18 hr at 37°C in a humidified 5% CO_2 in air atmosphere, cell viability was estimated by the ability of cells to reduce the chromogen 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium blue (MTT; Sigma). MTT (6 mg/ml) was added to each well and the plates were incubated for 4 hr at 37°C. After removal of the medium, cells were lysed with 100 μl of 0.006 M HCl in 2-propanol, and an equal volume of distilled, deionized H_2O was added. The amount of reduced MTT was measured spectrophotometrically at 570/690 nm. Recombinant human TNFα (Chiron) was used as a standard. One TNFα unit was defined as the dilution of sample required to produce 50% killing. The sensitivity of the assay was ≈1 pg/ml of plasma.

Murine IL-6 activity was determined by proliferation of the B.9 hybridoma cell line (modified from ref. 31). Plasma samples were serially diluted 1:40 to 1:320 (2-fold steps) in 96-well microtiter plates, and ≈3000 B.9 cells were added per well. Cells were cultured for 92 hr at 37°C in a humidified 5% CO_2 in air atmosphere. Cell viability was quantitated using MTT per TNFα above. Recombinant murine IL-6 (courtesy of P. Sehgal, The Rockefeller University) was used as a positive control. One IL-6 unit/ml was defined as the dilution of sample required to produce half-maximal B.9 proliferation. The sensitivity of the assay was ≈5 units/ml of plasma. Specificity of the assay was confirmed by the ability of polyclonal rabbit anti-recombinant murine IL-6 antisera to neutralize plasma bioactivity.

Physiologic Measurements. Carcass protein and lipid were determined gravimetrically (28). Eviscerated carcasses were frozen in liquid nitrogen and pulverized with solid carbon dioxide in a blender. Pulverized carcasses were then dried to a constant mass at 80°C. Lipid content was determined by sequential chloroform/methanol (1:1), ethanol/acetone (1:1), and diethyl ether extractions. Fat-free dry mass was used as an estimate of carcass protein content.

Plasma corticosterone was determined by radioimmunoassay (32). The sensitivity of the assay was 1 ng/ml.

The acute-phase protein amyloid P and albumin were measured by rocket immunoelectrophoresis (33). Briefly, 5 μl of plasma was added to punched circular wells in a 1% agarose gel containing 0.75% rabbit anti-murine amyloid P antiserum (Calbiochem) or 0.5% rabbit anti-murine albumin antiserum (Cappel Laboratories) and electrophoresed for 18 hr at 200 V in Veronal (pH 8.4) buffer. Nonspecifically bound proteins were removed by blotting followed by washing in 0.9% NaCl. Immunoprecipitates were visualized by staining the gels with 0.2% Coomassie brilliant blue. The quantities of amyloid P and albumin were determined by comparison of the heights of the "rockets" with those of commercial standards (Calbiochem).

Hematocrits were measured by microcentrifugation. Total leukocyte counts were determined with a Coulter Counter.

Statistics. Differences in cytokine, corticosterone, acute-phase protein, and hematologic responses measured repeatedly over the initial 72 hr were assessed by one- and two-way analysis of variance (time and antibody treatment). Intergroup comparisons were performed using Newman–Keuls multiple range test. One- and two-way analysis of variance and Newman–Keuls post-hoc comparison were also employed to assess differences in food intake, body weight, and carcass compositional changes in other studies.

RESULTS

In the present report, s.c. hindlimb administration of turpentine in the C57BL/6 mouse resulted in decreased food intake, rapid weight loss, leukopenia, hypoalbuminemia, and an increase in the hepatic acute-phase protein, amyloid P. IL-1 was not observed in the plasma of healthy mice, but detectable levels were observed 12–72 hr following induction of a

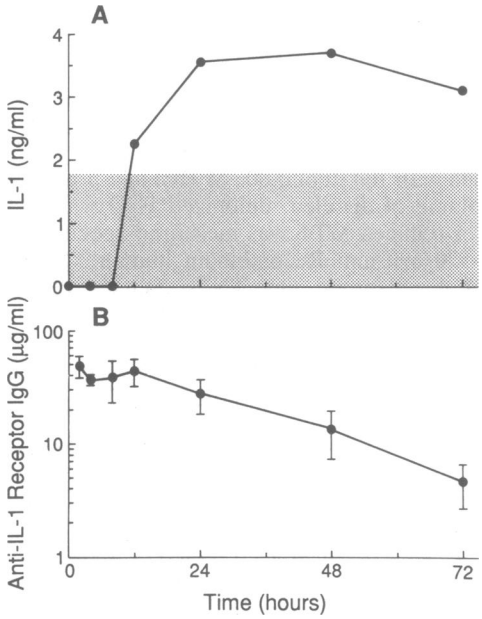

FIG. 1. Plasma IL-1 and IL-1 neutralizing activity. (*A*) Plasma IL-1 activity was determined by a competitive receptor-ligand binding assay (29). Sensitivity of the assay on 5-fold diluted plasma was 1.8 ng/ml. (*B*) Plasma clearance of anti-IL-1R mAb following i.p. administration 6 hr prior to induction of a turpentine-induced sterile abscess. Time zero represents the time of turpentine challenge (i.e., 6 hr after antibody administration). Each point represents the mean ± SEM of four mice.

turpentine-induced sterile abscess (Fig. 1*A*). Although TNFα was not detected in the circulation of any animals (data not shown), IL-6 concentrations peaked 8–12 hr following induction of the turpentine abscess, and levels remained elevated for 48 hr (Fig. 2*A*). In addition, plasma amyloid P levels increased 8–12 hr following induction of the turpentine abscess and peaked between 24 and 72 hr (Fig. 2*B*). Turpentine-treated mice developed a leukopenia within 2 hr, which was maintained over the 72-hr study period (Fig. 3*A*). Plasma albumin (Fig. 2*C*) and hematocrit (Fig. 3*B*) also declined.

When mice were passively immunized with 200 μg of rat anti-IL-1R mAb 6 hr prior to the induction of a turpentine-induced sterile abscess, peak levels of anti-IL-1R mAb were detected in the circulation within 6 hr (Fig. 1*B*). Concentrations remained at peak levels for 12–18 hr and thereafter exhibited a first-order exponential clearance with an estimated half-life of 19 hr. After 3 days, plasma concentrations had declined to 5 ± 3 μg/ml.

Several components of the acute-phase response were significantly attenuated by a single passive immunization of anti-IL-1R mAb prior to the turpentine abscess. Peak plasma IL-6 concentrations were diminished ($P < 0.01$) by ≈50% (Fig. 2). Plasma concentrations of the positive acute-phase reactant amyloid P were also reduced ≈50% ($P < 0.01$) by IL-1 receptor blockade versus nonimmune IgG-treated controls. In contrast, other components of the acute-phase response were unaffected by passive immunization against the IL-1 receptor. For example, plasma albumin levels declined in control and IL-1R mAb-immunized mice following turpentine challenge, reaching a nadir ≈24 hr following turpentine administration (both $P < 0.01$ vs. $t = 0$ hr). Anti-IL-1R blockade did not attenuate the increase in circulating corticosterone levels following turpentine treatment (Fig. 3). Hypoalbuminemia, leukopenia, and decreases in hematocrit were also unaffected by anti-IL-1R mAb pretreatment.

In contrast to passive immunization against the IL-1 receptor, pretreatment with polyclonal anti-murine TNFα antiserum failed to prevent increases in IL-6 and amyloid P observed during turpentine abscess formation (data not shown). In addition, anti-TNFα antiserum had no effect on the body weight, leukocyte count, hematocrit, or corticosterone changes seen following turpentine administration (data not shown).

Because anti-IL-1R mAb was rapidly cleared from the circulation (Fig. 4), food intake and body weight changes were evaluated over a 6-day period in mice that received daily passive immunizations. Daily injections of turpentine-challenged mice with 200 μg of anti-IL-1R mAb blocked the loss in body weight usually observed following induction of a turpentine abscess (Fig. 4*A*). Turpentine-challenged mice pretreated with anti-IL-1R mAb exhibited a small weight gain on the first day ($P < 0.05$) and by day 3 paralleled the weight change of uninjured controls. Body composition analysis confirmed that carcass fat and lean tissue (protein) were spared in turpentine-treated mice receiving anti-IL-1R mAb

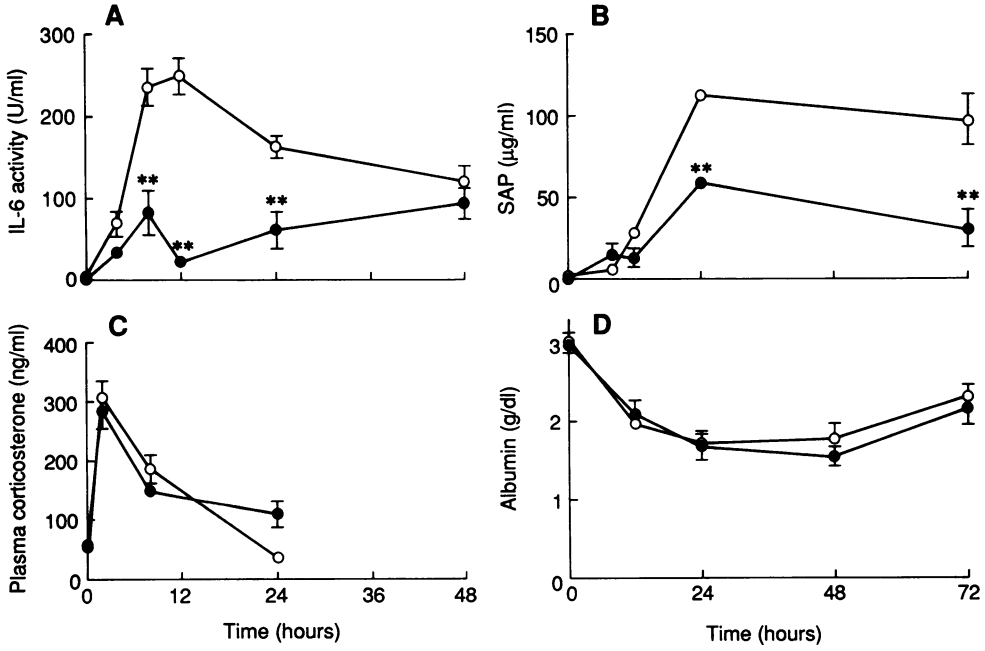

FIG. 2. Effect of s.c. turpentine injection on plasma IL-6 (*A*), plasma amyloid P (SAP, *B*), plasma albumin (*C*), and plasma corticosterone (*D*) levels in the presence (●) or absence (○) of anti-murine IL-1R mAb. Female C57BL/6 mice were pretreated with either 200 μg of rat anti-IL-1R mAb or nonimmune rat IgG (control) 6 hr prior to the inflammatory stimulus. Each point represents the mean ± SEM of four to eight mice. Data were analyzed by one- and two-way analysis of variance and post-hoc comparisons performed using Newman–Keuls multiple range test (**, $P < 0.01$).

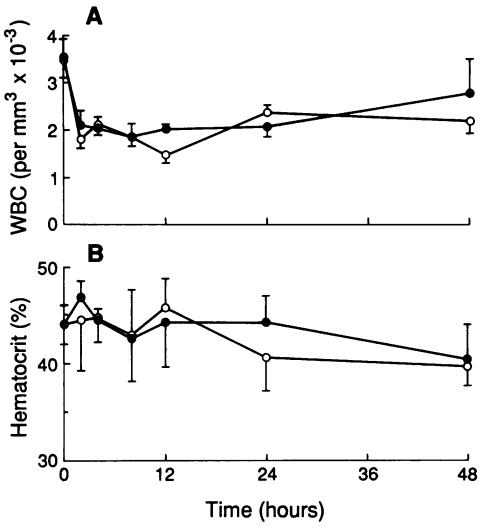

FIG. 3. Effect of s.c. turpentine injection on leukocyte counts (WBC, *A*) and hematocrit (*B*) in the presence (●) or absence (○) of anti-murine IL-1R mAb. Methods are as described in the legend to Fig. 2.

(Fig. 5). Preservation of body fat and protein could be partially explained by increasing food intake (Fig. 4*B*). Improvements in food intake following passive immunization were greatest 1 day after turpentine challenge and were maintained until the third study day.

DISCUSSION

Anorexia, weight loss, and the acute-phase response are common manifestations of inflammatory disease. However, the underlying mechanisms that regulate food intake, preservation of lean tissue, and the induction of hepatic acute-phase protein synthesis are complex and multifactorial. Despite the complexity of the cytokine network and the macrohormonal responses that occur during inflammation, the present study demonstrates that several components of this response are dependent upon the actions of a single cytokine, IL-1.

In previous *in vivo* studies, cytokines have been administered to healthy animals and subsequent tissue responses have been evaluated. Findings from such studies have suggested that several cytokines, including IL-1, TNFα, IL-6, and others, have the capability to induce anorexia, weight loss, and components of the acute-phase response (10, 13, 14, 34). The apparent similarity of actions among several cytokines has made it difficult to ascribe to individual proteins specific tissue responses. In the present report, however, the actions of IL-1 during inflammatory stress have been blocked using a neutralizing monoclonal antibody directed against the IL-1 receptor. This technique has identified those host responses that occur during inflammation that require IL-1. In this inflammatory model, the anorexia, weight loss, carcass protein and fat losses, and plasma IL-6 and amyloid P responses are dependent upon endogenous IL-1 activity. In contrast, hypoalbuminemia, leukopenia, and hypercorticosteronemia were not influenced by blockade of the T-cell/fibroblast type IL- receptor.

TNFα, like IL-1, is a proximal inflammatory mediator and is involved in the host inflammatory response (11). However, when similar turpentine-challenged mice were passively immunized with polyclonal antiserum against murine TNFα, body weight loss and carcass protein and fat depletion were not prevented. Similar passive immunization regimens employing the same antiserum preparation confer survival against lethal endotoxemia and inhibit tumor growth (28).

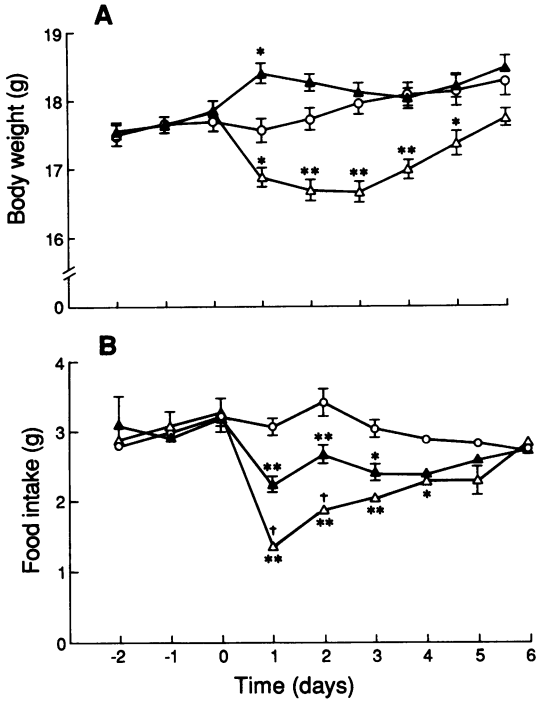

FIG. 4. Effects of s.c. turpentine injection on body weight (*A*) and food intake (*B*) in the presence (▲) or absence (△) of anti-murine IL-1R mAb. Uninjured mice received daily injections of saline and served as controls (○). Each point represents the mean ± SEM of 20 mice for body weight measurements and five cages of 4 mice each for food intake determinations. Data were analyzed by one- and two-way analysis of variance and post-hoc comparisons performed using Newman–Keuls multiple range test (*, $P < 0.05$; ** $P < 0.01$ vs. uninjured controls; †, $P < 0.01$ vs. anti-IL-1R mAb).

Whereas IL-1 and TNFα can induce anorexia, weight loss, and carcass protein and lipid depletion when administered exogenously (34), the food intake and body compositional changes induced by an endogenous cytokine response in this inflammatory model are more likely mediated by IL-1 rather than TNFα.

The failure of anti-IL-1R mAb to block other components of the host response to injury presumably mediated by IL-1, including hypercorticosteronemia and hypoalbuminemia, may be partly explained by the presence of at least one additional class of IL-1 receptor that is immunologically distinct from the EL-4 T-cell receptor (26). A 68-kDa IL-1 receptor has been identified on neutrophils, B lymphocytes, and monocytes and demonstrates a similar affinity for IL-1α

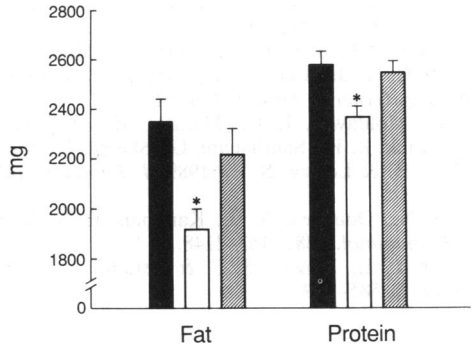

FIG. 5. Effect of s.c. turpentine injection on carcass fat and protein in the presence (diagonal bars) or absence (blank bars) of anti-murine IL-1R mAb. Uninjured mice (solid bars) served as controls. Each bar represents the mean ± SEM of 20 mice (*, $P <$ 0.05 vs. uninjured control and anti-IL-1R mAb).

and IL-1β (26). The failure to abrogate some of the inflammatory responses may be due to binding of IL-1 to this additional class of IL-1 receptor or to the influence of other mediators exhibiting similar biological actions.

We are unable to resolve whether inhibition of IL-1 is directly responsible for the increased food intake, maintenance of body weight, and decreased plasma amyloid P or whether these changes result from a diminished secondary cytokine response that is induced by IL-1. IL-6 and IL-1 induce the synthesis of several hepatic acute-phase proteins (35, 36). IL-1 receptor blockade decreased the plasma concentration of IL-6, suggesting that attenuation of the amyloid P response may have resulted from lower IL-6 levels. Conversely, the data suggest that the hypercorticosteronemia observed following turpentine administration does not directly regulate either the amyloid P, food intake, or body weight changes *in vivo*, since IL-1 receptor blockade significantly altered these responses in the absence of any change in circulating corticosterone.

It is presently unclear whether IL-1 blockade will prove beneficial to outcome. Although continued anorexia, weight loss, and persistent lean tissue catabolism during chronic illness contribute to morbidity (2–4), mobilization of body fat and lean tissue during inflammation may acutely provide the needed substrates and hormonal milieu for optimal tissue responses aimed at recovery. Many actions of IL-1 may be essential to host defenses (37); administration of IL-1 prior to or concurrent with bacterial infection increases antimicrobial activity and improves survival (38–40). In preliminary studies, McIntyre *et al.* (41) have reported that IL-1 receptor blockade increased mortality during bacterial infection in mice. Inhibition of IL-1 during acute inflammation may lead to suppressed immune surveillance and antimicrobial function.

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