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The Biological Activity *in Vivo* of Recombinant Murine Interleukin 1 in the Rat (42338)

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Abstract. The present study summarizes the biological response of rats to infusion with recombinant murine IL-1 (rIL-1) cloned in *Escherichia coli.* Thirty-seven male rats (135–180 g) were infused over a 6-hr period with either 0.008 *M* guanidine hydrochloride (the vehicle) or *E. coli* product (both groups are controls) or 1000, 3750, 7500, 15,000, or 37,500 LAF units/hr of rIL-1. The controls and the group receiving 1000 LAF units/hr of rIL-1 did not exhibit a change in body temperature during the experiment. A mild fever was noted with 3750 LAF units/hr which became significantly elevated with 7500 and 15,000 LAF units/hr. At a dose of 37,500 LAF units/hr of rIL-1 (in 0.08 *M* guanidine hydrochloride) the rats became hypothermic and died. An equivalent dose of guanidine hydrochloride alone (0.08 *M*) was not fatally toxic although the rats did become hypothermic. Plasma zinc levels were significantly depressed and white blood cell count elevated at 6 hr postinfusion onset. Resting energy expenditure (REE) was significantly depressed during an infusion of 7500 and 15,000 LAF units/hr of rIL-1 despite a concurrent elevation in body temperature. Whole-body leucine kinetics were unchanged by infusion with rIL-1. Plasma fibrinogen and serum haptoglobin and copper levels were not altered by rIL-1. In conclusion, murine rIL-1 is similar to monocytic-derived IL-1 in that it produces a fever, hypozincemia, and leukocytosis; however, rIL-1 does not induce changes in protein metabolism.

Biologically derived Interleukin 1 (IL-1) produced by activated monocytes and macrophages exhibits heterogeneity in terms of its relative molecular weight and charge properties. Recent studies suggest that human and murine IL-1 are synthesized as a large molecular weight (mol wt) precursor (ca. 33,000) that is cleaved to a low mol wt form (ca. 17,000) (1, 2). Isoelectric focusing gradients have identified several *p*I values in the range of 4.5–5.5 and 6.8–7.4 (3, 4).

Administration of monocytic derived IL-1 consisting of primarily *p*I = 7 and mol wt = 17,000 induces fever, trace metal redistribution, proliferation of certain leukocyte subpopulations, enhanced acute phase protein synthesis and an increased skeletal muscle catabolism (4). In addition, IL-1 administration also has been shown to induce an increase in resting energy expenditure (REE) (5); Tocco-Bradley *et al.,* unpublished observations) and whole-body amino acid flux, oxidation, and protein synthesis (6).

The characterization and utilization of biologically derived IL-1 are limited by lack of sufficient quantities of pure product that are not contaminated with IL-2, interferon, and endotoxin. With the recent cloning and expression of the IL-1 gene, however, quantities of recombinant murine IL-1 (rIL-1) are now available to study its *in vivo* effects (7). The goal of the present study was to characterize the *in vivo* response of rats to infusion with murine IL-1 cDNA cloned in *Escherichia coli* (*p*I = 5, mol wt = 17,000) obtained from Hoffmann–LaRoche Laboratories.

Materials and Methods. *Animal preparation.* Thirty-seven specific pathogen-free male Sprague–Dawley rats (135–180 g) were obtained from Taconic Farms (Germantown, N.Y.). Prior to the experiment, rats were housed two to a cage and maintained on a 12/12 hr light/dark photoperiod at an ambient temperature of 27 ± 1°C. Tap water and rodent chow (Charles River D-3000, Agway Agricultural Products, Minneapolis, Minn.) were provided *ad libitum.* Two days prior to the actual experiment, a Silastic catheter (0.025 × 0.04 in, medical grade; Dow Corning Corporations, Midland, Mich.) was inserted through the internal jugular vein into the superior vena cava of the rats under ether anesthesia. The tubing was tunneled subcutaneously and exited through a stab wound in the

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skin of the midscapular region. The externalized tubing was passed through a Teflon button and a stainless-steel spring, and attached to a flow-through swivel (Instech Laboratories, Philadelphia, Pa.) that permitted rats to move freely in their cages. The animals were kept on a continuous saline drip (2.5 ml/hr) in order to keep the lines patent. The rats were allowed to consume laboratory chow and tap water *ad libitum*. After a 2-day period, the animals were reweighed and only those animals that had maintained body weight and appeared healthy were used in the experiment.

Determination of body temperature. Body temperature was measured by battery-operated biotelemetry devices (Mini-Mitter, Inc, Sun River, Oreg.) implanted intraperitoneally at the time of cannulation. Output (clicks) from each transmitter was monitored by an AM radio receiver held outside each cage. The rate of discharge of each transmitter was proportional to the temperature of its environment; the time elapsed for 50 clicks from each transmitter (to the nearest 0.01 sec) was determined with a stopwatch. A temperature calibration curve established for each transmitter before implantation allowed calculation of the body temperature of each animal. Body temperature was measured every 15 min for the first hour and every 30 min for the subsequent 6-hr infusion period.

rIL-1, L-[1-^{14}C]leucine. [1-^{14}C]Leucine (50 mCi/mmole, ICN, Irvine CA) was added to the sera–saline infusate to estimate rate of whole-body leucine appearance, oxidation, and incorporation into protein, as well as skeletal muscle, liver, and seromucoid protein fractional synthetic rate (8).

The mouse rIL-1 was provided at a concentration of 2.0×10^6 LAF units/ml in 5 *M* guanidine hydrochloride by Hoffmann-LaRoche laboratories (7). The protein concentration of the rIL-1 was .33 mg/ml (6×10^6 LAF units/mg protein). For experimental use, the rIL-1 was diluted in physiological saline containing 0.1% normal rat sera (sera–saline) to varying concentrations to achieve infusion of 1000, 3750, 7500, 15,000, or 37,500 LAF units/hr (where infusion rate was 2.34 ml/hr). Control solutions consisted of either 0.008 *M* guanidine hydrochloride in sera–saline (the guanidine concentration equivalent to that infused with 7500 LAF units/hr) or a non-rIL-1 recombinant protein made in *E. coli* and purified in an identical manner to the mouse rIL-1. A Limulus lysate assay performed on the rIL-1 and non-rIL-1 proteins showed similar amounts of endotoxin which were negligible for the purpose of *in vivo* infusions. The rIL-1 and non-rIL-1 control infusates contained between 0.25 and 5.0 endotoxin units/ml (ca. 200 endotoxin units per μg of endotoxin) where 2.4 ml were infused over a 1-hr period.

Experimental design. On Day 3 following surgery, rats were transferred from their stainless-steel suspension cages to metabolic chambers that permitted collection and analysis of expired breath. Prior to beginning the infusion the animals were randomly assigned to one of seven groups and allowed to adapt to the metabolic chambers for 1 hr. Rats were then infused over a 6-hr period at a volume rate of 2.34 ml/hr through a syringe pump (+1%, Harvard Apparatus Co., Harvard, Mass.)

Group 1 ($n = 8$) served as controls, receiving a bolus injection of their infusate, immediately before a 6-hr continuous infusion either of 0.008 *M* guanidine ($n = 5$) in sera–saline or of the *E. coli* product ($n = 3$), in sera–saline, and 1 μCi/hr of [1-^{14}C]leucine. The rats receiving 0.008 *M* guanidine and *E. coli* product were considered as one control group since no significant differences were found in their responses.

Group 2 ($n = 5$) received a bolus intravenous infusion of 1000 LAF units of rIL-1 in sera–saline. For the subsequent 6-hr group 2 received 1000 LAF units/hr dissolved in sera–saline containing 1 μCi/hr of [1-^{14}C]leucine.

Group 3 ($n = 6$) received a bolus intravenous injection of 3750 LAF units of rIL-1 in sera–saline. For the subsequent 6 hr group 3 received 3750 LAF units/hr dissolved in sera–saline containing 1 μCi/hr of [1-^{14}C]leucine.

Group 4 ($n = 7$) received a bolus intravenous injection of 7500 LAF units of rIL-1 in sera–saline. For the subsequent 6 hr group 4 received 7500 LAF units/hr dissolved in sera–saline containing 1 μCi/hr of [1-^{14}C]leucine.

Group 5 ($n = 6$) received a bolus intravenous injection of 15,000 LAF units of rIL-1 in sera–saline. For the subsequent 6 hr group 5 received 15,000 LAF units/hr dissolved in sera–saline containing 1 μCi/hr of [1-^{14}C]leucine.

Group 6 ($n = 2$) received a bolus intravenous injection of 37,500 LAF units of rIL-1 in sera–saline. For the subsequent 6 hr group

6 received 37,500 LAF units/hr in sera-saline containing 1 μCi/hr [1-^{14}C]leucine.

Due to the finding that 37,500 LAF units/hr was toxic to rats, another control group was added to receive an infusion of guanidine in sera–saline at a concentration equal to that in the 37,500 LAF units/hr, i.e., 0.08 *M* guanidine. Hence, group 7 ($n = 3$) received a bolus dose of 0.08 *M* guanidine in sera-saline followed by a 6-hr infusion of 0.08 *M* guanidine in sera–saline containing 1 μCi/hr of [1-^{14}C]leucine.

During the 6-hr continuous infusion room air was circulated through the metabolic chambers at a rate of 1.6 liters/min using a Masterflex pump (Cole-Palmer Instruments Co., Chicago, Ill.). At hourly intervals the [^{14}C]carbon dioxide was trapped in scintillation vials containing absolute ethanol, phenolpthalein (0.1% w/v), and hyamine hydroxide (Packard Instrument Co., Downers Grove, Ill.). Ten milliliters of commercial scintillant (Betaflour, National Diagnostics, Somerville, N.J.) was added to the sample and analyzed for total ^{14}C radioactivity by using a Beckman LS-8000 liquid scintillation spectrometer (Beckman Instruments, Fullerton, Calif.)

At Hours 2 and 4, O2 and CO2 expired breath concentrations were measured with Beckman OM-11 polarigraphic oxygen and LB-2 infared carbon dioxide analyzers (Beckman, Schiller Park, Ill.). Prior to the 2-hr mark the pump air flow rate was determined over a 15-min period so that *V*O2 and *V*CO2 could be calculated. Before each analysis, the analyzers were calibrated with primary standard gases (5% CO2, 12% O2, Corning Medical, Medfield, Mass.).

At the end of the 6-hr continuous infusion the animals were sacrificed by decapitation and the blood was collected in heparinized tubes. The body was quickly dissected, removing the liver and portions of the rectus abdominus muscle. The liver was weighed and a 1-g piece was placed in 4 ml of 10% sulfosalicylic acid (SSA) and completely frozen in liquid nitrogen to halt all metabolic processes. A 1-g piece of muscle was similarly frozen. Another gram of liver and of muscle was frozen in saline for total nitrogen measurements. All samples were stored at −25°C until analysis.

In a second experiment, 12 additional rats were given a bolus injection of 15,000 LAF units of rIL-1 (a dose known to be pyrogenic) to determine the acute phase protein response. At 12 hr postinjection animals were bled by cardiac puncture in order to determine plasma fibrinogen levels and serum haptoglobin and copper levels.

Analytical methods. *V*O2, *V*CO2, respiratory quotient, and nonprotein resting energy expenditure were obtained by indirect calorimetric calculations (9).

Whole-blood leukocyte counts were determined manually using a hemocytometer and light microscopy. The remaining blood was centrifuged at 3500 rpm for 15 min, following which the plasma was removed and stored at −25°C. The plasma was later thawed for determinations of leucine specific activity, seromucoid specific activity and zinc concentrations.

Plasma was diluted 1:5 with double distilled water for zinc analysis. Plasma was deproteinized with 20% trichloroacetic acid and diluted twofold for copper analysis. Plasma zinc and copper concentrations were determined with an atomic absorption spectrophotometer (Perkin-Elmer Corp., Norwalk, Conn.). Sample concentrations were determined by comparing absorbance values with a standard curve (10).

Fibrinogen levels were determined by Dade Thrombin Clotting Time. Haptoglobin levels were determined by fluorescence light scattering using a human antibody to haptoglobin.

One milliter of plasma was deproteinized with 0.2 ml of 30% SSA for measurement of plasma leucine concentration and specific activity. The deproteinized serum was vortexed, incubated at 25°C for 30 min, and centrifuged for 15 min at 4000 rpm. Six-tenths of a milliliter of the supernatant was further deproteinized with 0.2 ml of 30% H_2O_2. This was incubated at 37°C for 1 hr and at 70°C for 30 min. Following the incubation, and centrifugation, 150 μl was analyzed for total leucine content using a D-400 amino acid analyzer (Dionex Corporation, Sunnyvale, Calif.). Two hundred and fifty milliliters was placed in a scintillation vial with 5 ml of commercial scintillant (Instagel, United Technologies Packard, Downers Grove, Ill.), and analyzed for total ^{14}C radioactivity with a Beckman LS-8000 liquid scintillation spectrometer. To calculate the plasma specific activity the following equation was used:

[^{14}C]LEU (dpm/ml)/[LEU] (μmole/ml)

= SA LEU (dpm/μmole)

where [^{14}C]LEU is the plasma [^{14}C]leucine activity expressed in dpm/ml, [LEU] is the plasma leucine concentration, and SA LEU is the plasma specific activity of leucine.

Rate of whole body leucine appearance were estimated from

Q (μmole/hr)

= IN (dpm/hr)/SA max (dpm/μmole)

where Q is the appearance, IN, the isotopic infusion rate and SA max, the plasma specific activity. It was assumed that a plateau labeling (steady state) of the plasma compartment was achieved when the SA max was reached in the expired breath (between 2 to 3 hr of continuous L-[^{14}C]leucine infusion) (11).

The percentage of leucine appearance oxidized (% LEU OX) was determined by dividing the appearance of ^{14}CO2 at plateau (OUT) by the infusion rate of [1-^{14}C]leucine (IN). The oxidation rate of leucine was then calculated by multiplying the appearance (Q) by the percentage oxidized.

The incorporation of leucine into whole body protein was subsequently derived from the difference between leucine appearance and oxidation, and the leucine release from whole-body protein breakdown was derived from the difference between appearance and intake.

The tissues, liver, and rectus abdominus muscle, were broken down into bound (SB) and intracellular (SI) proteins by SSA for analysis and determination of the fractional synthetic rates. The precipitate, SB, was washed three times with 2% SSA, and after being dried, 20- to 40-mg samples were either solubilized in quaternary ammonium hydroxide (Soluene-350; Packard Co., Downers Grove), added to 10 ml of commercial scintillant (Betaflour) and analyzed for 14C radioactivity with a Beckman LS-8000 liquid scintillation spectrometer, or spectrophotometrically analyzed for total nitrogen after a micro-Kjeldahl digestion. One hundred and fifty milliliters of the tissue supernatant, SI, was analyzed by HPLC (Waters Associates, Milford, Mass.) for total leucine content. An additional 1 ml was further deproteinized with 0.2 ml 30% H_2O_2, centrifuged, and 0.5 ml of the supernatant was removed to be analyzed for ^{14}C radioactivity. The SI was mixed with commercial scintillant.

The protein synthesis rates in the liver, rectus abdominus muscle, and the seromucoid fractions were derived from the equation of Garlick *et al.* (12) as previously described (13).

Statistical analysis. Data are expressed as means ± standard error of the means (SEM). The data from groups 1 through 5 was compared for statistical differences using an analysis of variance (ANOVA) and the Tukey test for multiple comparisons.

Results. *Febrile response.* The febrile response over 6 hr in rats infused with increasing doses of rIL-1 is shown in Fig. 1. The groups receiving 7500 and 15,000 LAF units/hr exhibited significant febrile responses by 4-hr postinfusion onset as compared to controls. An explanation for the biphasic nature of the fever response with 7,500 LAF units/hr is not apparent. The animals infused with 3750 LAF units/hr failed to show a significant change in body temperature from controls although body temperature was elevated 0.8°C by 3-hr postinfusion onset. An infusion of 37,500 LAF units/hr appeared to be toxic. The rats became hypothermic rapidly and died by 5 hr postinfusion onset. In order to ascertain if the toxicity was due to the dose of rIL-1 or to the high molarity of guanidine hydrochloride infused with the rIL-1, another group was infused with 0.08 M guanidine hydrochloride that did not contain the rIL-1. This group also become hy-

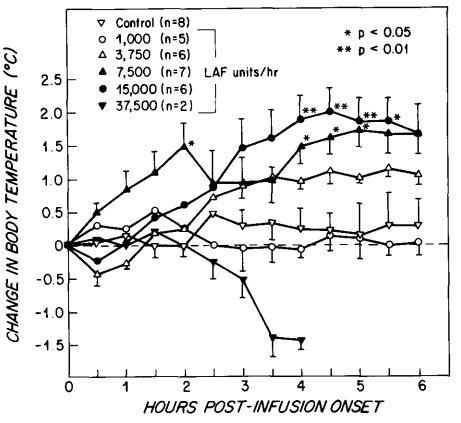

FIG. 1. Change in body temperature of rats influsediv over 6 hr with either 0.008 M guanidine hydrocholoride or *E. coli* products (controls), 1000, 3750, 7500, 15,000, or 37,500 LAF units/hr of rIL-1. Values represent means ± SEM. *$P < 0.05$, **$P < 0.01$ compared to controls.

pothermic and lethargic looking although they were not nearly as ill and did not die. It appears that the combination of the high dose of rIL-1 and guanidine hydrochloride together was fatally toxic to the rats.

Energy expenditure. Figure 2a summarizes the resting energy expenditure (REE) measured at 2 and 4 hr. The groups infused with 7500 and 15,000 LAF units/hr of rIL-1 had significantly depressed REE as compared to controls. Interestingly, body temperature was significantly elevated at these same time points (Fig. 2b) with the exception being at 2-hr postinfusion onset in the group infused with 15,000 LAF units/hr.

Zinc and white blood cell count. Plasma zinc levels at 6-hr postinfusion onset were significantly lower than controls ($P < 0.01$) in the groups infused with 7500 and 15,000 LAF units/hr (44 and 49%, respectively) (Fig. 3a). Rats receiving an infusion of rIL-1 at a dose of 7500 and 15,000 LAF units/hr exhibited white blood cell counts that were greater than control values by 41 and 58%, respectively (Fig. 3b). These differences were significantly different from control by Student's *t* test but not by the Tukey test for multiple comparisons.

Protein dynamics. Table I summarizes the whole-body leucine kinetics measured following the infusion of varying doses of rIL-1. The appearance rates of leucine in the plasma compartment were similar between the control group and each of the rIL-1 infused groups. Whole-body leucine oxidation and incorporation into whole-body protein were also similar in all the groups.

Protein synthesis rates of the rectus abdominus muscle and hepatic nonsecretory fraction were unchanged in rats infused with varying doses of rIL-1 (Table II).

In order to characterize the acute phase protein response to rIL-1, plasma fibrinogen and serum haptoglobin and copper were compared at 12 hr postinjection between controls and rats receiving a bolus injection of 15,000 LAF units/hr of rIL-1 (Table III). Approximately 95% of serum copper normally exists tightly bound to ceruloplasmin (14); thus, increases in serum copper which attend the acute phase response reflect an increase in ceruloplasmin. The rIL-1 did not induce an increase in these acute phase proteins or copper levels.

Discussion. Recombinant murine IL-1 demonstrates both similarities and dissimilarities with monocytic-derived or biologically derived IL-1. Among the metabolic alterations induced by both rIL-1 and monocytic-derived IL-1 are fever, hypozincemia, and leukocytosis. The only observable difference in the pyrogenic response induced by rIL-1 was a more delayed onset in the rise in body temperature (Fig. 1) than previously reported for monocytic derived human IL-1 (13). Specifically, a latent fever onset was observed in rats receiving 3750 and 15,000 LAF units/hr of rIL-1. Rats receiving 7500 LAF units/hr displayed a biphasic fever response to this inter-

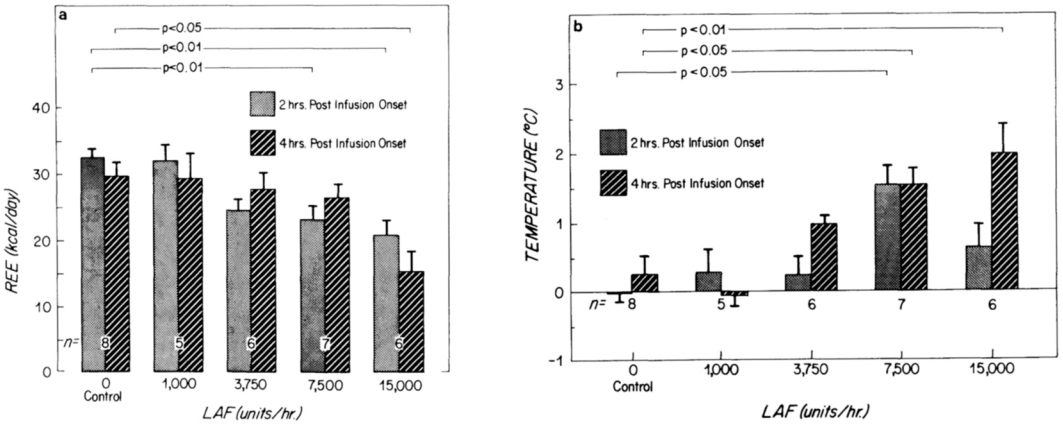

FIG. 2. (a) Resting energy expenditure (REE) and (b) change in body temperature from baseline at 2- and 4-hr postinfusion onset in rats infused iv over 6 hr with either 0.008 *M* guanidine hydrocholoride or *E. coli* product (controls), 1000, 3750, 7500, or 15,000 LAF units/hr or rIL-1. Values represent means ± SEM.

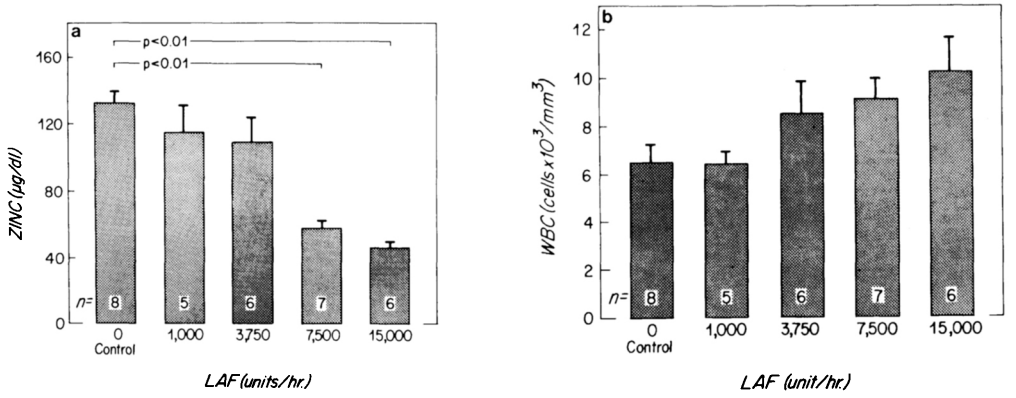

FIG. 3. (a) Plasma zinc concentration and (b) white blood cell count at 6-hr postinfusion onset in rats infused over 6 hr with either 0.008 *M* guanidine hydrocholoride or *E. coli* product (controls), 1000, 3750, 7500, or 15,000 LAF units/hr of rIL-1. Values represent means ± SEM.

mediate dose of rIL-1 (i.e., 7500 LAF units/hr) which can not be attributed to endotoxin, since all the rIL-1 doses were made from a common concentrate of rIL-1. In addition, as a control for endotoxin, we injected another group of rats with a similar concentration of a non-rIL-1 recombinant protein made in *E. coli* and purified in an identical manner to the mouse rIL-1. This group was similar to the 0.008 *M* guanidine hydrochloride controls in their responses and was therefore included as part of the control group for statistical comparisons. Consistent with other metabolic responses to monocytic derived IL-1 (15), plasma zinc levels fell and white blood cell count rose by approximately 40–50% in the rIL-1 infused animals.

However, murine recombinant IL-1 lacks the ability to induce certain other biological responses which normally occur with monocytic-derived IL-1. We have previously reported a significant rise in REE following infusion with monocytic-derived IL-1 (5); Tocco-Bradley *et al.*, unpublished observations). Unexpectedly, REE was actually depressed following infusion of rIL-1 despite a significant increase in body temperature (Fig. 2). This finding is perhaps the most remarkable difference noted between the rIL-1 and the biologically derived IL-1. The usual mechanism of fever is to increase heat production by means of muscle contraction (shivering) and by metabolic processes or nonshivering thermogenesis that includes the energy-requiring processes of gluconeogenesis and protein synthesis and oxidation. In conjunction with acceleration in heat production is a reduction in heat dissipation by alteration in

TABLE I. WHOLE BODY LEUCINE KINETICS FOLLOWING INFUSION WITH rIL-1

Group	Mean + SEM rate (µmole 100 g body wt per hr)			
	Appearance	Oxidation	Incorporation into protein	Release from protein degradation
Control (n = 8)	57.1 ± 4.7	8.3 ± 1.1	48.8 ± 4.4	57.1 ± 4.7
1000 units (n = 5)	59.5 ± 4.9	9.3 ± 0.8	50.2 ± 5.4	59.5 ± 4.9
3750 units (n = 6)	63.1 ± 9.9	8.4 ± 1.4	54.7 ± 8.8	63.1 ± 9.9
7500 units (n = 5)	57.9 ± 3.4	8.0 ± 1.0	50.0 ± 3.0	57.9 ± 3.4
15,000 units (n = 6)	59.2 ± 6.6	8.7 ± 1.5	50.5 ± 5.4	59.2 ± 6.6

TABLE II. PROTEIN SYNTHESIS IN TISSUES FOLLOWING INFUSION WITH rIL-1

Group	Mean ± SEM synthesis rate (% per day)	
	Rectus abdominus muscle	Hepatic nonsecretory fraction
Control (n = 8)	2.3 ± 0.2	31.5 ± 4.6
1000 units (n = 5)	2.5 ± 0.2	33.4 ± 4.5
3750 units (n = 6)	2.8 ± 0.6	32.2 ± 4.1
7500 units (n = 6)	1.9 ± 0.2	34.4 ± 1.4
15,000 units (n = 6)	2.0 ± 0.2	32.9 ± 2.3

blood flow to the skin. In order for the host to elevate body temperature while decreasing REE as found in the present study using rIL-1, the conservation of heat must be dramatically increased over the normal situation. This finding provides evidence that energy expenditure and body temperature may have separate control mechanisms. The effect of the rIL-1 on REE may prove to have some future therapeutic value in the septic or injured host who is hypermetabolic and thus hypercatabolic if rIL-1 could reduce energy expenditure and with it protein catabolism. Furthermore, since rIL-1 does produce some of the same beneficial responses seen with biologically derived IL-1, such as white blood cell elevation and trace mineral depression, rIL-1 might be the preferred immunostimulant (15) if these effects could be achieved without an increase in energy expenditure or protein catabolism.

The host protein changes in response to human monocytic-derived IL-1 can be characterized by an increase in both the anabolic and catabolic state of the organism. Accelerated proteolysis in muscle (16) and increased amino acid appearance and incorporation into protein (6, 13) may play a role in redistribution of body protein stores to the liver and subsequent acute phase protein synthesis. Consistent with this, biologically derived IL-1 also stimulates an increase in seromucoid and hepatic nonsecretory protein synthesis (6, 13). Using the identical methodology we have shown that rIL-1 does not stimulate similar changes in protein synthesis, breakdown, or oxidation. Our finding that protein metabolism is not stimulated is consistent with the fact that REE was not increased following rIL-1 infusion.

The liver's metabolic alterations during the acute phase response and in response to monocytic-derived IL-1 include the rise in concentration of a large number of plasma proteins of hepatic origin, which are collectively called the acute phase proteins. Following an injection of rIL-1 we did not see an increase in the acute phase proteins, haptoglobin and fibrinogen, which normally rise two- to fourfold by 12 to 24 hr following administration of IL-1 (17, 18). In addition, the copper binding acute phase protein ceruloplasmin was not increased as indicated by the unchanged copper levels. Evidence that changes in serum copper reflect changes in ceruloplasmin includes the fact that whenever plasma copper and ceruloplasmin are measured concomitantly, both exhibit parallel changes (19). Although haptoglobin, fibrinogen, and copper levels did not change it is possible that other acute phase proteins such as α_1-acid glycoprotein might increase in response to rIL-1. Alternatively, the general acute phase protein response may occur with a more latent onset than 12 hr postinjection in response to rIL-1 as compared to monocytic-derived IL-1.

Until now, IL-1 isolated from various cells and tissues by different methods has displayed a heterogeneity of molecular weights and pI values. It was believed that the various forms of IL-1 were derived from a single gene, and that variations in proteolytic modification of the IL-1 result in the diversity of its molecular forms (1, 20). Multiple biological effects on various tissues have been reported for this family of biologically derived IL-1 molecules.

The diversity of IL-1 may be better understood with the recent expression and cloning

TABLE III. ACUTE PHASE PROTEIN RESPONSE WITH INFUSION OF rIL-1

Group	Fibrinogen (mg/dl)	Haptoglobin (mg/dl)	Copper (μg/dl)
Control (n = 6)	43.5 ± 5.4	265.8 ± 9.8	188.6 ± 19.7
rIL-1 (n = 6)	52.5 ± 3.4	285.7 ± 5.1	206.8 ± 7.4

Note. Values represent means ± SEM.

of IL-1 (7, 21). It is becoming increasingly clear that more than one gene may be coding for the different IL-1 molecules of similar molecular weight (21). Lomedico and co-workers (7) reported the cloning sequence analysis and expression of murine IL-1 cDNA in *E. coli.* They demonstrated that the 17K rIL-1 ($pI = 5$) is the carboxyterminus of the 33K precursor and that the carboxyterminal 156-amino acid fragment has very high activity for thymocyte proliferation.

With the availability of this newly cloned murine rIL-1, it was of particular interest to us to explore a wide range of possible biological activities of the rIL-1 expressed and cloned by Lomedico and co-workers (7). We found that this murine rIL-1 molecule has apparent biological similarities and differences from the monocytic-derived IL-1 previously reported on. Although the murine rIL-1 is pyrogenic and causes hypozincemia and leukocytosis, it does not increase energy expenditure or protein catabolism or anabolism. Our data provide strong evidence that this murine rIL-1 is not the identical molecule to the monocytic-derived IL-1 whose biological activities have been extensively outlined in the past. It is also possible that the rIL-1 differs from the monocytic-derived IL-1 in that the rIL-1 represents a pure protein expressed by one gene while the semipurified monocytic-derived IL-1 may actually represent a family of proteins each responsible for different or overlapping biological activities. Finally, rIL-1 may lack certain activities due to a partial denaturation or incorrect renaturation during the recombinant process. This possibility is highly unlikely since the murine rIL-1 still exhibited a variety of activities including fever, hypozincemia, leukocytosis, and *in vitro* LAF activity (7). Future experiments with the various forms of cloned IL-1 now being produced will contribute to our understanding of this family of hormones collectively called IL-1.

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