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Effects of Continuously Administered Murine Interleukin-1α: Tolerance Development and Granuloma Formation

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Continuous infusion of murine recombinant interleukin-1α (rIL-1α) into rats by using intraperitoneally implanted osmotic pumps led to marked decreases in body weight, liver enzymes (serum glutamic oxalacetic transaminase, serum glutamic pyruvic transaminase, and sorbitol dehydrogenase), appetite, and mobility and increases in drinking, blood urea nitrogen, and total peripheral blood leukocytes within 3 days. Granuloma formation was found in the local area of rIL-1α release. As early as day 3, a focal infiltrate of polymorphonuclear leukocytes, mononuclear leukocytes, and plasma cells filled the area; by day 6, extensive fibrosis was found. A loss of rIL-1α-induced changes, with the exception of granuloma formation, occurred by day 10. A marked decrease in the response to rIL-1α was also observed when animals were challenged by implantation of new pumps containing rIL-1α, with monitoring of body weight, or by subcutaneous injection of rIL-1α, with monitoring of serum colony-stimulating factor production. We propose that, even in the continuous presence of interleukin-1, replacement of the acute responses to interleukin-1 by restoration of more normal physiology may be advantageous upon acquisition of specific immunity.

Interleukin-1 (IL-1) plays a role in both immune (T-cell) responsiveness to antigenic stimulation (11, 39) and low-grade, chronic granulomatous diseases (7). In addition, IL-1 produces infiltration of neutrophils into rabbit (23) and mouse (13) skin, is chemotactic for human neutrophils (32) and lymphocytes (20), and promotes adhesion of both neutrophils and lymphocytes to vascular endothelium (3). Synovial dendritic cells have been shown to produce IL-1 (9), and IL-1 induces fibroblast proliferation (27). Moreover, IL-1 has been found in inflammatory joint fluid (45) and is thought to play an important part in the pathogenesis of rheumatoid arthritis (8, 17, 21, 36).

IL-1 is also a primary mediator of the acute-phase response to inflammation, infection, and injury (6) and may regulate a large number of processes throughout the body via a central homeostatic mechanism (28). The onset of acute infection, fever, sepsis, traumatic injury, and burns is accompanied by skeletal muscle catabolism (muscle wasting), resulting in a marked negative nitrogen balance (1). Acute infection is also accompanied by the suppression of food intake, which may also contribute to weight loss (29). Intraperitoneal (i.p.) injection of either IL-1 (19) or tumor necrosis factor (TNF) (37, 40) has been shown to produce a decrease in food intake in rats. In addition, continuous infusion of murine recombinant interleukin-1α (rIL-1α) for several days has been shown to induce a decrease in food consumption with attendant weight loss, decreased mobility, and increased drinking behavior (25). Moreover, IL-1 has been shown to induce rapid metabolism of brown adipose tissue (5a).

IL-1 has thus been reported to have multiple specific local in vivo effects. On the other hand, it has been suggested that the role of IL-1 is hormonal in nature. Therefore, by continuous infusion, we have sought to determine which of the activities of IL-1 is more dominant.

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MATERIALS AND METHODS

IL-1. Murine rIL-1α was cloned and purified from *Escherichia coli* by the molecular genetics group at Pfizer Central Research (G. O. Daumy, J. M. Merenda, A. S. McColl, G. C. Andrews, A. E. Franke, K. F. Geoghegan, and I. G. Otterness, Biochim. Biophys. Acta, in press). The biological activity of rIL-1α was verified in the lymphocyte-activating factor assay, by induction of colony-stimulating factor (CSF) and cartilage resorption in vitro, and by induction of serum amyloid P and CSF in vivo. Endotoxin was determined to be present at less than 1 part in 100,000 by the *Limulus* assay. Moreover, direct infusion of endotoxin (lipopolysaccharide W; *E. coli* O111:B4) (Difco Laboratories, Detroit, Mich.) at 10 times its highest concentration in rIL-1α produced no effects on our measured parameters.

Continuous infusion. Alzet osmotic minipumps (1.0 μl/h for 7 days or 0.5 μl/h for 14 days) (Alza Corp., Palo Alto, Calif.) containing either murine rIL-1α dissolved in phosphate-buffered saline (PBS) or PBS alone were implanted into the abdominal cavities of male Sprague-Dawley rats (250 to 300 g; Charles River Breeding Laboratories, Inc., Wilmington, Mass.) as previously described (25). The concentration of rIL-1α with which the osmotic pump was filled resulted in an effective infusion rate of 3.0 μg per day.

Animals with implanted osmotic minipumps were weighed immediately following implantation. Body weight was then measured periodically over the course of 14 days.

Serum chemistry and hematology. Animals were bled on days 0, 3, and 10. Serum was collected and analyzed for levels of serum glutamic oxalacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), sorbitol dehydrogenase (SDH), and blood urea nitrogen (BUN) on a Cobas-Bio automatic analyzer. Urine was collected on day 10, and protein was determined on a Cliniteck 200 automatic analyzer. For hematologic determination, animals were bled on days 0, 2, 7, and 14, and total leukocyte counts were made on an Ortho ELT8 automatic cell counter.

Behavioral measures. Eating, drinking, and locomotor

activity were measured in individual electronically monitored behavioral chambers as previously described (25, 35). Briefly, eating (eats) and drinking (licks) were measured as the amount of time contact was made with the metal wire food bin and the water spout, respectively. Each eat corresponds to a contact time of 1/12 of a second; each lick corresponds to a contact time of 1/60 of a second. Horizontal locomotor activity (crossovers) was measured as the number of crossovers from one quadrant of a chamber to another. Vertical locomotor activity (rears) was measured as the number of times contact was made with a metal strip located 7 cm above the floor on all four sides of the box. Cages were maintained on a standard 12-h-on, 12-h-off light cycle (i.e., lights off at 4:00 p.m. and lights on at 4:00 a.m.). Rats were placed in the behavioral chambers 10 days after minipump implantation, at 2:30 p.m. Data were recorded continuously over the next 24 h (day 11 of rIL-1α administration).

Data were broken down into 2-h intervals, and the mean for each animal group was plotted as a function of time. The data were also examined as a block of 24 h subjected to analysis of variance followed by Dunnett's *t* test for comparison of multiple-treatment groups to a single control group.

Light microscopy. Pieces of the fibrotic mesentery covering osmotic minipumps were excised, fixed in 10% phosphate-buffered Formalin (pH 7.4), processed according to standard histological techniques, and embedded in paraffin. Sections were cut at 5 μm on a Leitz 1512 rotary microtome and stained with hematoxylin and eosin. Light micrographs were taken on a Zeiss photomicroscope II at ×160 with Kodak Plus-X black-and-white print film.

Anti-rIL-1α antibody titer. Serum from animals continuously infused with rIL-1α was collected at day 14. Animals were also rechallenged at day 21 with 1.0 μg of rIL-1α, and serum was collected at day 28. Positive control animals were sensitized subcutaneously (s.c.) with 100 μg of rIL-1α emulsified in complete Freund adjuvant, and serum was collected at 21 days.

Anti-rIL-1α antibody titers were determined by an enzyme-linked immunoabsorption assay. Briefly, 80 ng of rIL-1α in 100 μl of pH 8.4 borate-buffered saline was added to each well of Immulon 1 (Nunc, Roskilde, Denmark) 96-well polystyrene plates. Plates were incubated overnight at 4°C and washed three times with PBS containing 0.05% Tween 20. To block nonreactive sites on the wells, plates were incubated in 3% bovine serum albumin for 2 h at 37°C before being used.

Dilutions of antisera in 100-μl aliquots were added to wells coated with rIL-1α. Plates were incubated at 37°C for 1 to 2 h and washed. Beta-galactosidase-conjugated goat anti-rat immunoglobulin (Fisher Scientific Co., Medford, Mass.) diluted in PBS was added to each well in 100-μl aliquots. Plates were again incubated at 37°C for 1 to 2 h. The bound enzyme was developed by the addition of 1.0 mg of *o*-nitrophenyl-β-D-galactopyranoside per ml in pH 7.5 substrate buffer containing 0.01 M Tris base, 0.1 M sodium chloride, 0.01 M magnesium chloride, and 0.1 M 2-mercaptoethanol. Color development was measured on a Dynatech MR 700 microtiter reader at a wavelength of 405 nm.

Induction of serum CSF. Rats continuously infused with either PBS or rIL-1α at 3.0 μg per day were challenged with a s.c. injection of 500 ng of rIL-1α on day 6 and bled 2 h postchallenge, and the serum was collected.

The presence of CSF in the serum was determined by its ability to induce and maintain the proliferation of normal mouse C3H/HeN bone marrow cells in vitro, as measured by tritiated thymidine ([^{3}H]TdR) incorporation. The assay used was a modification of the procedure of Prystowsky et al. (30). Briefly, 0.1 ml of McCoy 5A medium containing 15% fetal calf serum, 20 U of penicillin-streptomycin per ml, and 10^{-4} M 2-mercaptoethanol was placed in each well of a 96-well flat-bottom microdilution plate. Rat serum (30 μl) and 10^5 mouse bone marrow cells in 0.1 ml were added in succession. Plates were incubated at 37°C in 5% CO_2 and high humidity for 3 days. [^{3}H]TdR (50 μl of a 50-μCi/ml solution) was added to each well, and plates were incubated for 18 h at 37°C in 5% CO_2 and high humidity. Samples were harvested on an LKB cell harvester. Filters were washed, dried, and counted on a liquid scintillation counter. The mean counts per minute ± standard error for three samples for each test serum were determined.

In addition, the presence of CSF in the rat serum samples was verified by its ability to induce granulocyte and/or monocyte colonies from normal rat bone marrow cells in soft agar. The method of Bradley and Metcalf (4) was used. Briefly, 10^5 normal rat bone marrow cells were plated along with 30 μl of test serum in 1.0 ml of McCoy 5A medium plus 15% fetal calf serum, 20 U of penicillin-streptomycin per ml, and 0.3% Noble agar in 35-mm culture dishes. After 10 days of culturing at 37°C in 5% CO_2 and high humidity, the numbers of granulocyte and/or monocyte colonies of ≥50 cells were counted by using an ×20 dissecting microscope. The mean numbers for three plates for each serum sample were determined.

RESULTS

Effect of subchronic administration of rIL-1α on body weight. Our previous findings (25) indicated that animals subacutely infused with rIL-1α undergo a marked weight loss after 2 to 3 days of continuous administration and return only to preinfusion weight by day 7. We have here determined the effects of 14-day subchronic infusion with rIL-1α. As previously reported, weight loss appeared to plateau around day 4. Thereafter, the animals gained weight. After day 7, the effects of rIL-1α appeared to be lost, accelerated weight gain occurred, and the animals attained normal body weight by days 12 to 14 (Fig. 1). In contrast, animals infused with PBS underwent only a modest weight loss within the first 24 h and thereafter continuously gained weight.

Loss of effects of rIL-1α on serum chemistry and hematology parameters. The effects of continuous administration of rIL-1α on serum chemistry parameters were examined. Results for the five parameters SGOT, SGPT, SDH, BUN, and urinary protein are shown in Table 1. On day 3, rIL-1α induced decreases in the liver enzymes SGOT, SGPT, and SDH, each of which is considered a measure of liver function, and an increase in BUN, which is considered a measure of protein catabolism. On day 10, these parameters had all returned to normal. Continuous infusion of PBS produced no changes in these parameters at day 3 or 10. Urinalysis for animals continuously exposed to rIL-1α revealed no increase in protein on days 3 and 10 over the level in animals continuously exposed to PBS.

The effects of continuous administration of rIL-1α on total leukocyte number were also determined. By day 7, the leukocyte number in animals continuously infused with rIL-1α was found to be double that in animals continuously infused with PBS (Fig. 2). (The IL-1-induced increase in leukocyte number was selective for neutrophils; data not shown.) On day 14, however, the total leukocyte number in animals infused with rIL-1α had returned to the vehicle

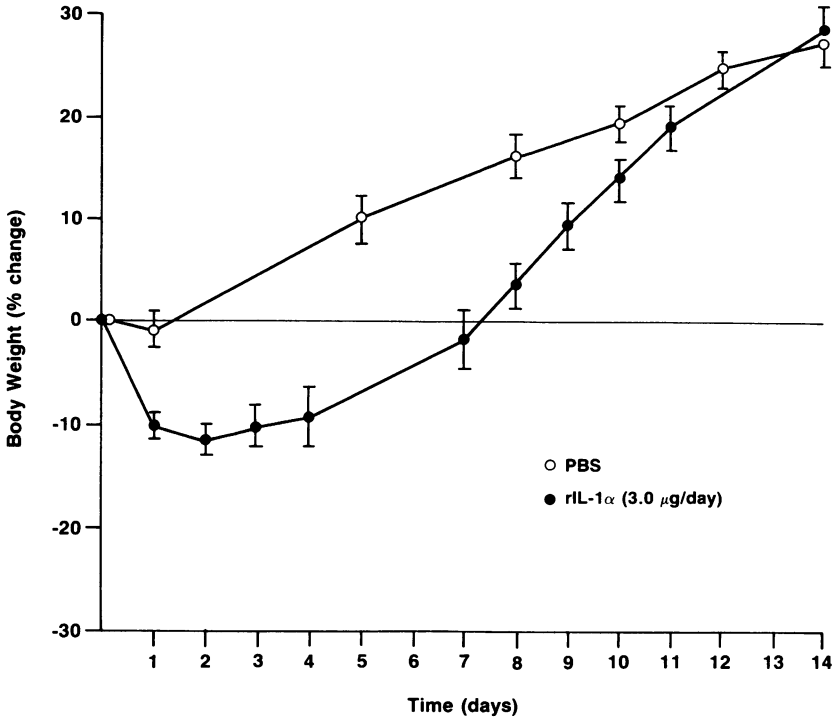

FIG. 1. Effect of rIL-1α on body weight. Animals exposed to rIL-1α were compared with animals exposed to vehicle. The mean percent change in body weight ± the standard error during 14 days of continuous infusion at 3.0 μg per day was measured for five animals per group.

control level. Since the leukocyte number in both rIL-1α-infused and PBS-infused animals was still elevated over basal measurements taken on day zero, pump implantation must have had an effect unrelated to the substance being infused.

Loss of effect of rIL-1α on behavioral measures. In a previous study (25) we found marked decreases in eating (eats), horizontal locomotor activity (crossovers), and vertical locomotor activity (rears) and an increase in drinking (licks) from days 2 to 5 of continuous rIL-1α infusion. It was confirmed that on day 3 (Table 2) rIL-1α at 3.0 μg per day produced pronounced changes in 24-h total counts for each behavioral parameter, as previously reported (25). Measurements were made again on day 11 of treatment. All behavioral parameters in animals treated with rIL-1α had returned to the levels in the PBS-infused and normal controls at some point prior to day 11 (Table 2).

TABLE 1. Effect of rIL-1α on serum chemistry parameters

Parameter	Treatment	Level in serum (μg/ml) (mean ± S.E.) on the following day of administration[a]:		
		0	3	10
SGOT	PBS (control)	68.4 ± 5.1	73.4 ± 9.2	74.6 ± 8.8
	rIL-1α	72.6 ± 4.3	33.8 ± 2.3	93.0 ± 25.0
SGPT	PBS (control)	26.8 ± 1.6	35.4 ± 3.6	27.8 ± 3.1
	rIL-1α	28.4 ± 2.4	8.80 ± 2.2	31.8 ± 12.2
SDH	PBS (control)	3.40 ± 0.25	4.80 ± 0.59	5.60 ± 0.52
	rIL-1α	4.20 ± 0.20	1.20 ± 0.20	7.80 ± 1.8
BUN	PBS (control)	17.2 ± 1.4	18.8 ± 0.20	14.2 ± 0.30
	rIL-1α	16.8 ± 2.5	38.8 ± 5.0	19.6 ± 1.4
Urinary protein	PBS (control)	0–Tr	0–1+	0–1+
	rIL-1α	0–Tr	0–Tr	0–1+

[a] Continuous administration of rIL-1α at 3.0 μg per day. There were five animals per group.

Loss of effect of rIL-1α is not due to osmotic pump failure. Since our previous findings (25) showed that feeding and body weight in animals continuously infused with rIL-1α begin to return to normal during days 2 to 3 of administration, it was important to confirm that the osmotic pumps were still releasing rIL-1α at a point when body weight and feeding had begun to return to normal. Osmotic pumps (7-day) which had been implanted in one group of animals were removed after 3 days and reimplanted in a second group of animals. Weight loss after 3 days in the second group of animals was identical (13%) to that in the first group.

Fibrotic encapsulation of osmotic pumps. In experiments with 7-day osmotic pumps, it was noted at the end of the experiment that the pumps had become enclosed by a fibrotic connective tissue capsule which had formed within the mesentery supporting the intestines. The sequence of events during the development of this fibrotic capsule was studied by using a combination of necropsy and histologic evaluation.

Normal mesentery (day zero) is a thin sheet of loose connective tissue covered by mesothelium and containing bundles of collagen and elastic fibers, fat cells, and blood vessels (Fig. 3A). On day 3 of continuous rIL-1α administration, pumps were found to be loosely encapsulated. The layer of the adjacent mesentery just beneath the mesothelium was heavily infiltrated with a mixture of 30% polymorphonuclear leukocytes and 70% mononuclear leukocytes (Fig. 3B). Approximately 10 to 20% of the latter had the eccentrically placed nucleus characteristic of plasma cells. A

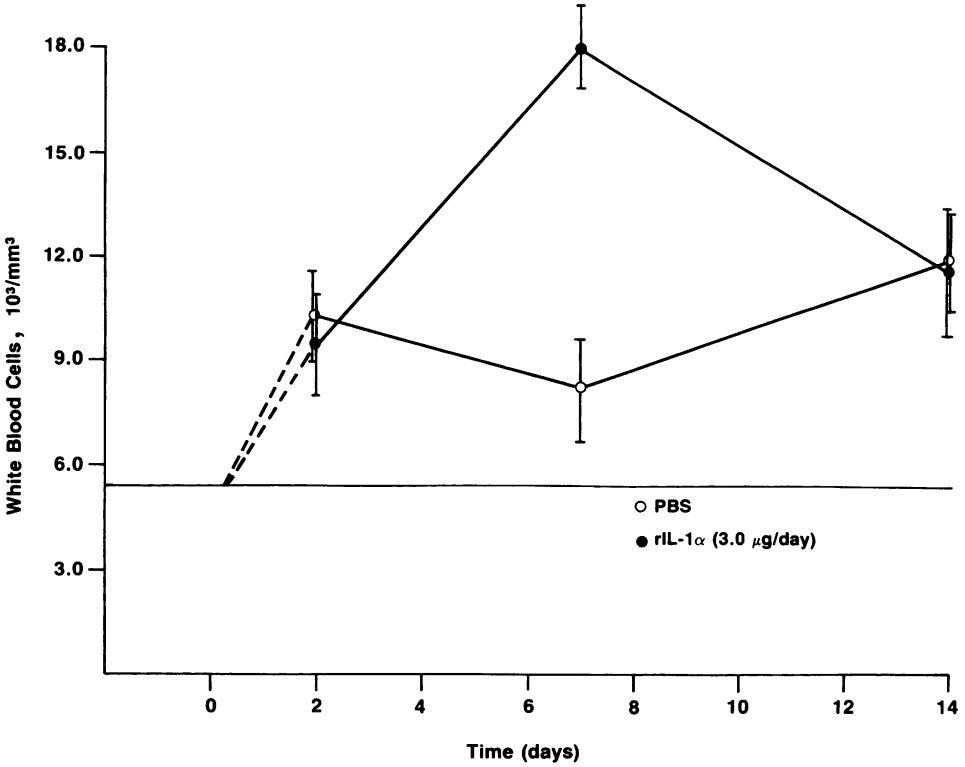

FIG. 2. Effect of rIL-1α on leukocyte number. Animals exposed to rIL-1α were compared with animals exposed to vehicle. The mean total leukocyte number ± the standard error during 14 days of continuous infusion at 3.0 μg per day was measured for three animals per group. The solid horizontal line represents the leukocyte numbers in control, nonimplanted animals.

small number of plump epithelioid fibroblasts was also present. Fine collagen fibrils were found in the spaces between infiltrated cells and fibroblasts. Below the outer layer, leukocyte infiltration was more diffuse. However, this area was characterized by extensive proliferation of endothelial cells (capillary buds), incipient proliferation of fibroblasts, and the deposition of thicker collagen fibers.

TABLE 2. Effect of rIL-1α on behavioral measures

Behavorial measure	Treatment	Continuous counts divided into 24-h-interval totals mean ± S.E. on the following day of administration[a]:	
		3	11
Eats	None	76.4 ± 5.9	63.1 ± 3.9
	PBS (control)	76.6 ± 6.3	57.6 ± 6.1
	rIL-1α	42.1 ± 11.2[b]	80.1 ± 10.4
Licks	None	41.7 ± 1.4	39.7 ± 1.4
	PBS (control)	49.9 ± 3.9	40.5 ± 3.5
	rIL-1α	80.5 ± 15.1[c]	47.7 ± 3.2
Crossovers	None	510 ± 135	370 ± 65
	PBS (control)	279 ± 24	282 ± 30
	rIL-1α	168 ± 22[c]	315 ± 26
Rears	None	194 ± 24	144 ± 14
	PBS (control)	157 ± 17	138 ± 17
	rIL-1α	67 ± 18[c]	166 ± 26

[a] Continuous administration of rIL-1α at 3.0 μg per day. There were eight animals per group. Eats and licks are given in thousands.
[b] $P < 0.05$ (Dunnett's t test).
[c] $P < 0.01$ (Dunnett's t test).

On day 6, pumps were found to be firmly encapsulated. Cell infiltration into the layer of the mesentery adjacent to the pumps was significantly resolved, as shown by the finding of pyknotic leukocyte nuclei (Fig. 3C). Moreover, viable infiltrated cells were now found within an extensive meshwork of collagen fibers. In the deeper layer, capillary proliferation was now nearly completely obliterated by extensive fibroblast proliferation and collagen fiber deposition.

Histologic examination of mesentery from animals continuously infused with PBS (data not shown) revealed a connective tissue architecture similar to that of normal mesentery and no significant foreign body reaction.

Loss of effect of rIL-1α is not due to fibrotic encapsulation of osmotic pumps. To determine whether the return of body weight and behavioral measures to normal was due to blockage by fibrosis of the release of rIL-1α into the peritoneal cavity, we carried out the following experiment. On day 10, animals which had fully regained the weight lost from prior i.p. implantation of 14-day pumps were reimplanted i.p. with a second set of pumps also releasing rIL-1α at 3 μg per day. Upon implantation of the second set of pumps, we did not even measure a loss of weight equivalent to that produced by a PBS-filled pump in a naive animal, and the animals continued to gain weight through day 14 (Fig. 4). In contrast, implantation of a second set of pumps releasing rIL-1α into animals exposed only to the PBS vehicle produced weight loss equivalent to that produced by rIL-1α in unexposed animals.

Loss of effect of rIL-1α is not due to fibrosis of the peritoneal cavity. Preliminary experiments showed that s.c. implantation of osmotic pumps releasing rIL-1α produced weight loss equivalent to that produced by i.p. implantation. Since the

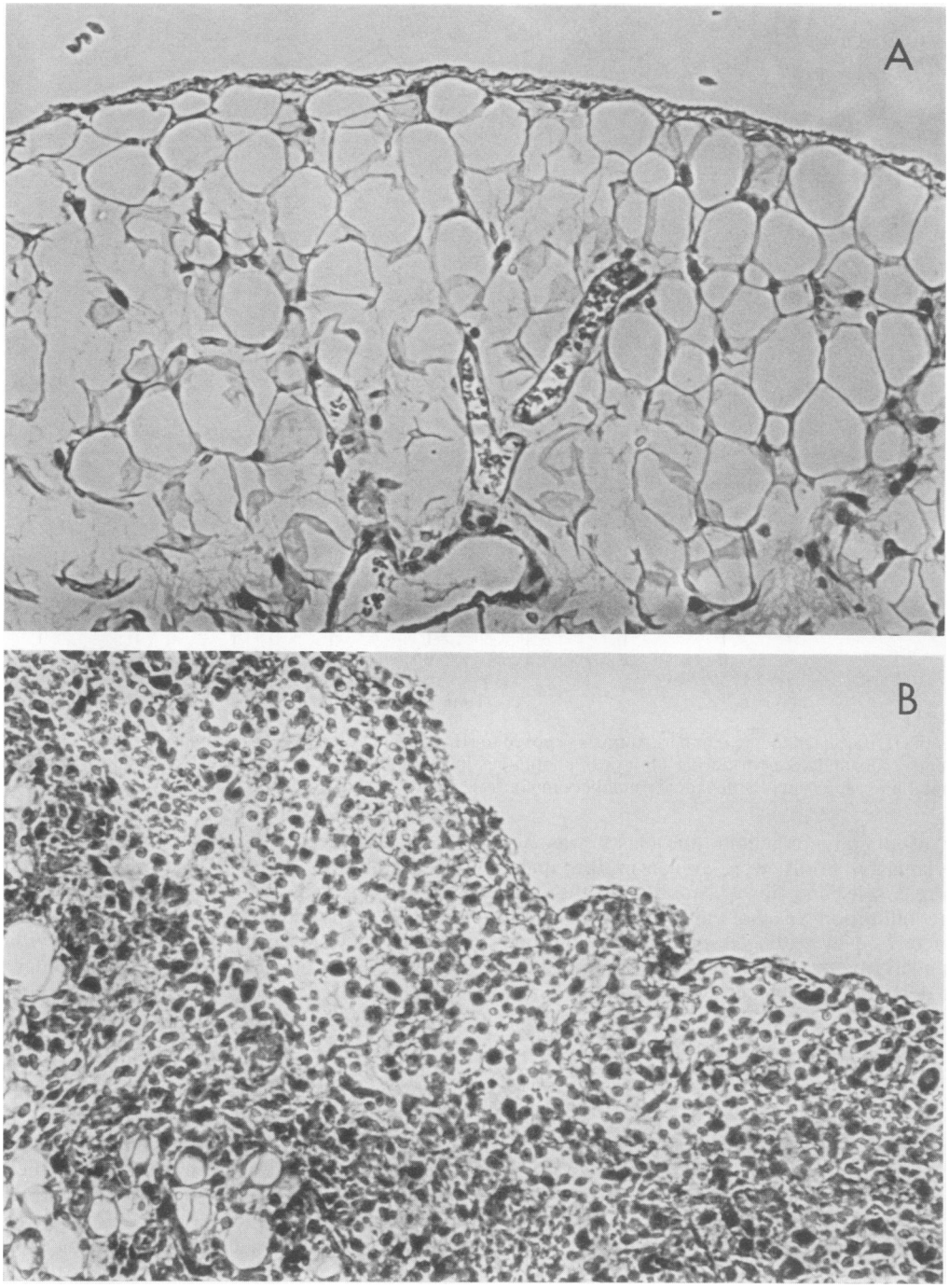

FIG. 3. Developmental sequence of fibrotic encapsulation of osmotic pumps. (A) Normal mesentery. (B) Mesentery after 3 days of continuous infusion of rIL-1α at 3.0 μg per day. (C) Mesentery after 6 days of continuous infusion of rIL-1α at 3.0 μg per day.

previous experiment did not preclude the possibility of a fibrotic walling off of the entire peritoneal cavity, 7-day osmotic pumps releasing rIL-1α at 3.0 μg per day were implanted i.p. on day 9 into animals which had regained the weight lost from prior s.c. implantation of 14-day osmotic pumps. In contrast to the findings in the previous experiment, after implantation of the second set of pumps, there was approximately a 3% drop from the day 9 prechallenge weight level (Fig. 5). However, the degree of weight loss, while significant, was modest in comparison with that produced in animals continuously exposed to PBS s.c. and was within the normal range produced by surgical implantation of PBS-filled pumps alone. In confirmation of the results of the previous two experiments, we also found reduced weight loss when the second set of pumps was implanted s.c. into animals exposed to rIL-1α i.p.

Loss of effect of rIL-1α is not due to production of anti-rIL-1α antibody. An anti-rIL-1α antibody enzyme-linked

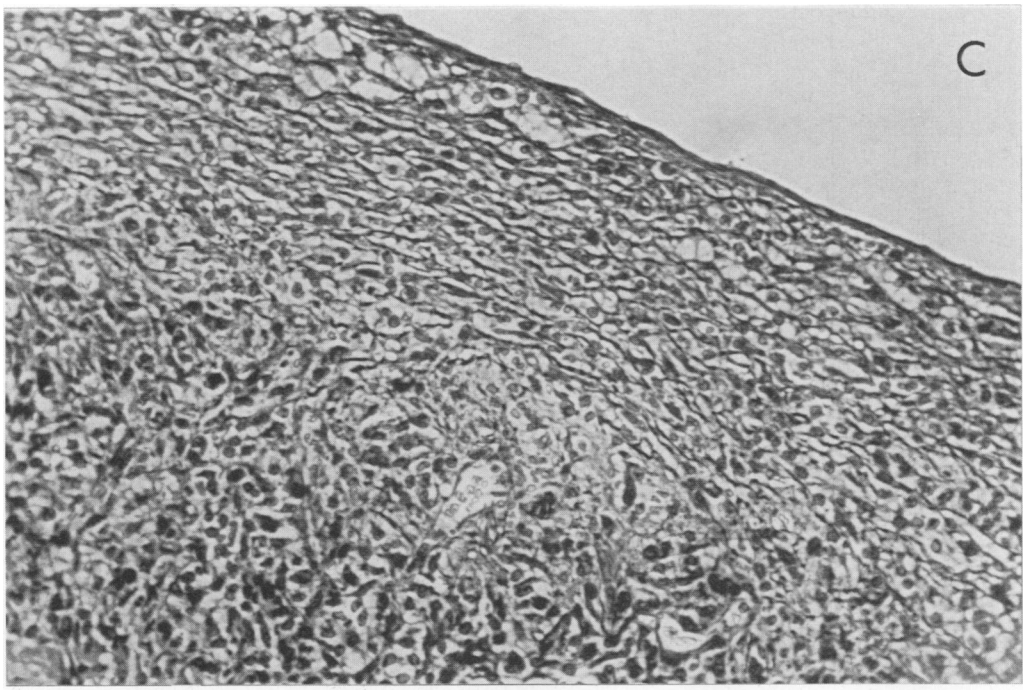

FIG. 3—*Continued*

immunoabsorption assay was used to compare serum from animals infused with rIL-1α for 14 days with serum from animals immunized for 21 days with 100 μg of rIL-1α emulsified in complete Freund adjuvant. The anti-rIL-1α antibody titers of serum from both untreated and PBS-infused animals were also determined. Animals immunized with rIL-1α in complete Freund adjuvant showed a significant anti-rIL-1α antibody titer (data not shown). In contrast, the titer in animals continuously infused with rIL-1α was indistinguishable from that in either untreated or PBS-

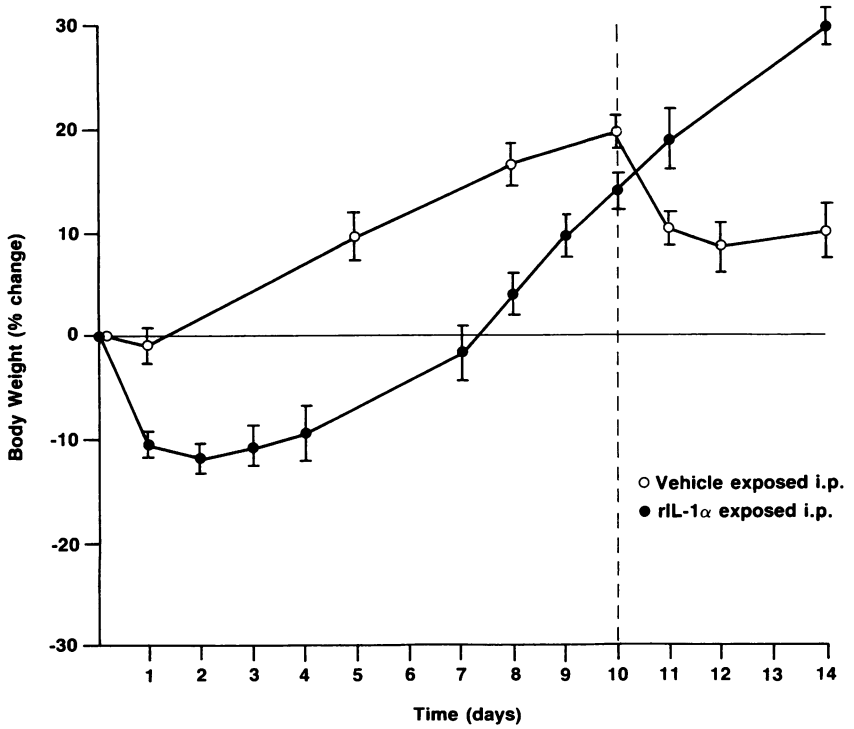

FIG. 4. Lack of effect of fibrotic encapsulation of osmotic pumps on the loss of the effect of rIL-1α on body weight. A second set of osmotic pumps releasing rIL-1α were implanted (dotted line) in animals exposed to rIL-1α i.p. and animals exposed to vehicle. The mean percent change in body weight ± the standard error during 14 days of continuous administration was measured for five animals per group.

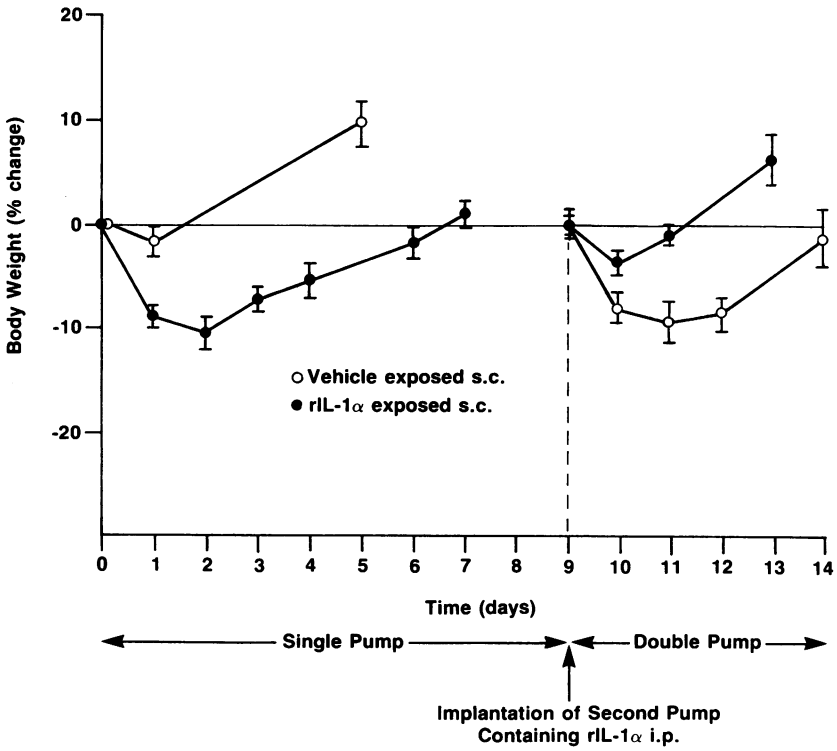

FIG. 5. Lack of effect of fibrosis of the peritoneal cavity on the loss of the effect of rIL-1α on body weight. A second set of osmotic pumps releasing rIL-1α were implanted in animals exposed to rIL-1α s.c. and animals exposed to vehicle. The mean percent change in body weight ± the standard error during 14 days of continuous administration was measured for five animals per group.

infused animals. A booster challenge of the rIL-1α-infused animals also failed to elicit a significant anti-rIL-1α antibody titer.

Tolerance of induction of CSF. As it is known that the administration of rIL-1α leads to enhanced levels of serum CSF (42), tolerance was also determined by examining the ability of rats to respond to rIL-1α with the synthesis of CSF. Serum from rats challenged s.c. with 500 ng of rIL-1α produced nearly a fivefold increase in [^{3}H]TdR incorporation in normal animals or animals infused with PBS (Table 3). In contrast, s.c. challenge with rIL-1α failed to produce a significant increase in CSF production in animals infused with rIL-1α, when compared with PBS-infused control animals.

CSF-inducing ability was verified by the generation of granulocyte and/or monocyte colonies by rat bone marrow cells in agar. Neither serum from animals infused with PBS nor serum from animals infused with rIL-1α at 3.0 μg per day was able to induce colony formation in agar. Serum from animals infused with PBS and challenged with 500 ng of rIL-1α induced a mean of 42 ± 2 colonies. In contrast, serum from animals infused and challenged with rIL-1α induced a mean of only 9 ± 3 colonies.

TABLE 3. Induction of CSF synthesis

Pretreatment[a]	Challenge[b]	Proliferation of mouse bone marrow cells ([^{3}H]TdR cpm)[c]	No. of rat bone marrow colonies in agar[c]
PBS	None	943 ± 125	0
	rIL-1α (500 ng)	4,442 ± 657	42 ± 2
rIL-1α (3.0 μg per day)	None	335 ± 154	0
	rIL-1α (500 ng)	774 ± 492	9 ± 3

[a] Continuous infusion for 6 days.
[b] S.C. injection on day 6; serum was collected 2 h later.
[c] Mean ± standard error. There was a minimum of three animals per group.

DISCUSSION

In a previous study (25) we described the effect of continuous infusion of rIL-1α in rats. We observed decreases in body weight and food consumption which were apparent within 1 day of beginning infusion and which returned to preinfusion levels by day 7. Here we confirm and expand on that study by reporting the effects of longer-term administration of rIL-1α on body weight, behavioral parameters, serum chemistry and hematology parameters, and local tissue morphology. Animals continuously infused with rIL-1α began to gain weight around day 5. Weight gain was accelerated as compared with that in controls, and by days 12 to 14 infused animals reached the same body weight as controls. In addition, the early decreases in liver enzymes (SGOT, SGPT, and SDH) and increases in BUN and total leukocyte number all returned to control levels by day 10. Since the elevation in BUN was not accompanied by an increase in urinary protein, continuous long-term infusion of rIL-1α did not apparently lead to muscle wasting.

We found no significant difference in eating or drinking behavior, horizontal locomotor activity, or vertical locomotor activity between animals infused with rIL-1α and controls on day 11 of continuous infusion, indicating that the previously reported decreases in appetite and mobility on day 3 (25) had been lost upon longer treatment. Therefore, like the effects on body weight, the behavioral effects

induced by infusion of rIL-1α all appear to be lost upon longer rIL-1α infusion.

We are intrigued by the rapidity and extent of fibrotic encapsulation of osmotic pumps. A slow release of IL-1 has been shown to induce chronic inflammatory granulomatous tissue in mice (10). In addition, IL-1 has been implicated as a primary mediator in granuloma formation to foreign bodies and schistosome eggs (5) and has been shown to potentiate pannus formation in a rat model of peptidoglycan-polysaccharide-induced arthritis (38). IL-1 is known to stimulate fibroblast proliferation (27, 31) and fibroblastic collagen synthesis (43). Thus, our findings confirm evidence which suggests that IL-1 may be a potent inducer of granuloma formation.

The finding that the early effects of rIL-1α on body weight, behavior, serum chemistry, and hematology had returned to normal indicated that there must be a compensatory mechanism which permits this recovery. However, before we could suggest that tolerance had occurred, it was important to rule out a number of trivial explanations: (i) failure of the osmotic pumps; (ii) blockage of the release of rIL-1α into the peritoneal cavity due to fibrotic encapsulation of osmotic pumps; (iii) failure of rIL-1α to be absorbed into the circulation due to fibrosis of the lining of the peritoneal cavity; and (iv) neutralization by anti-rIL-1α antibody. We carried out experiments to rule out each of these possibilities: reimplantation of the pump into another animal confirmed that the release of rIL-1α is continuous; insertion of a second pump at either fibrotic or nonfibrotic sites failed to elicit a normal rIL-1α response; and, finally, anti-rIL-1α antibodies were not detected.

We then sought to directly confirm tolerance by using the ability of the rats to respond to rIL-1α by inducing CSF production. Animals continuously infused with rIL-1α were found to be unresponsive to s.c. rIL-1α challenge on day 6. The response was suppressed to normal levels, as measured by [^{3}H]TdR incorporation, and colony formation was markedly suppressed. Although more colonies were found in rIL-1α-injected, rIL-1α-infused animals than in saline-injected, rIL-1α-infused animals, suggesting that tolerance was not absolute, the magnitude of this difference was much smaller than the difference in response to rIL-1α challenge between control PBS-infused and rIL-1α-infused animals.

The mechanism of tolerance of IL-1 cannot be determined from the results of this study. The rapid development of granulomas may help limit the systemic exposure to IL-1. However, another mechanism, such as receptor desensitization (18, 22) or induction of an IL-1 inhibitor (33, 34), would be required to explain the decreased response to a secondary challenge with IL-1.

Tolerance of the lethal effects of lipopolysaccharide is well known (2, 14, 16) and has recently been reported for IL-1 (44) and TNF (37, 44). Although IL-1 has been reported to induce the production of TNF (26), we were unable to find an elevation of TNF even during the initial stages of continuous infusion of rIL-1α, and administration of high-titer anti-TNF antibody failed to inhibit granuloma formation (15) or weight loss, confirming that the IL-1 effects were not secondary to TNF production (unpublished data). It seems reasonable that tolerance of the numerous systemic effects of IL-1 should develop during long-term exposure, and we propose that it may be a necessary requirement for survival of chronic infection. If the synthesis and release of IL-1 represent a first line of defense against acute infection (12, 24, 41), the acute systemic effects of this cytokine may be useful for rapid mobilization of the body's nonspecific (natural) defense system. However, if the infectious episode is of long duration, the systemic response may be too severe and may compromise host survival.

We found that it took 3 to 5 days to attain good tolerance of IL-1. This duration is close to the time it takes to detect the onset of specific immunity, as measured by splenic plaque formation or early delayed hypersensitivity. Thus, if IL-1 is a mediator of primary host resistance, the duration of mobilization of nonspecific resistance to an infectious insult is comparable to the time required to elicit a specific immune response. This may be important for optimizing the interplay between specific immunity and nonspecific immunity in the host defense against infection.

LITERATURE CITED

1. **Beisel, W. R.** 1975. Metabolic response to infection. Annu. Rev. Med. **26**:9–20.
2. **Beutler, B., N. Krochin, I. W. Milsark, C. Luedke, and A. Cerami.** 1986. Control of cachectin (tumor necrosis factor) synthesis: mechanisms of endotoxin resistance. Science **232**: 977–980.
3. **Bevilacqua, M. P., J. S. Pober, M. E. Wheeler, R. S. Cotran, and M. A. Gimbrone.** 1985. Interleukin-1 activation of vascular endothelium: effects on procoagulant activity and leukocyte adhesion. Am. J. Pathol. **121**:393–403.
4. **Bradley, T. R., and D. Metcalf.** 1966. The growth of mouse bone marrow cells *in vitro*. Aust. J. Exp. Biol. Med. Sci. **44**:287–300.
5. **Chensue, S. W., I. G. Otterness, G. I. Higashi, C. S. Forsch, and S. K. Kunkel.** 1989. Monokine production by hypersensitivity (*Schistosoma mansoni* egg) and foreign body (Sephadex bead)-type granuloma macrophages. J. Immunol. **142**:1281–1286.

5a. **Dascombe, M. J., N. J. Rothwell, B. O. Sagay, and M. J. Stock.** 1989. Pyrogenic and thermogenic effects of interleukin 1-β in the rat. Am. J. Physiol. **256**:E7–E11.

6. **Dinarello, C. A.** 1984. Interleukin-1 and the pathogenesis of the acute phase response. N. Engl. J. Med. **311**:1413–1418.
7. **Dinarello, C. A.** 1984. Interleukin-1: an important mediator of inflammation. Trends Pharmacol. Sci. **5**:420–422.
8. **Dinarello, C. A., and J. W. Mier.** 1986. Interleukins. Annu. Rev. Med. **37**:173–178.
9. **Duff, G. W., O. Forre, K. Waalen, E. Dickens, and G. Nuki.** 1985. Rheumatoid arthritis synovial dendritic cells produce interleukin-1. Br. J. Rheumatol. **5**(Suppl.):94–97.
10. **Dunn, C. J., M. M. Hardee, A. J. Gibbons, N. D. Staite, and K. A. Richard.** 1988. Interleukin-1 induces chronic granulomatous inflammation, p. 329–334. *In* M. C. Powanda, J. J. Oppenheim, M. J. Kluger, and C. A. Dinarello (ed.), Monokines and other non-lymphocytic cytokines. Alan R. Liss, Inc., New York.
11. **Durum, S. K., J. A. Schmidt, and J. J. Oppenheim.** 1985. Interleukin-1: an immunological perspective. Annu. Rev. Immunol. **3**:263–287.
12. **Gladue, R., A. Girard, and M. Newborg.** 1988. Enhanced antibacterial resistance in neutropenic mice treated with human recombinant interleukin-1 beta. Agents Actions **24**:130–136.
13. **Granstein, R. D., R. Margolis, S. B. Mizel, and D. N. Sauder.** 1986. *In vivo* inflammatory activity of epidermal cell-derived thymocyte activating factor and recombinant interleukin-1 in the mouse. J. Clin. Invest. **77**:1020–1027.
14. **Greisman, S. E., and R. B. Hornick.** 1976. Endotoxin tolerance, p. 43–50. *In* R. F. Beers and E. G. Bassett (ed.), The role of immunologic factors in infectious, allergic, and autoimmune processes. Raven Press, Publishers, New York.
15. **Kindler, V., A.-P. Sappino, G. E. Grau, P.-F. Piguet, and P. Vassalli.** 1989. The inducing role of tumor necrosis factor in the development of bactericidal granulomas during BCG infection. Cell **56**:731–740.
16. **Madonna, G. S., J. E. Petersen, E. E. Ribi, and S. N. Vogel.** 1986. Early-phase endotoxin tolerance: induction by a detoxified lipid A derivative, monophosphoryl lipid A. Infect. Immun. **52**:6–11.
17. **Malone, D. G., S. M. Wahl, G. Tsokos, H. Cattell, J. L. Decker,**

and R. L. Wilder. 1984. Immune function in severe, active rheumatoid arthritis. J. Clin. Invest. **74:**1173–1185.

18. **Matsushima, K., J. Yodi, Y. Tagaya, and J. J. Oppenheim.** 1986. Down-regulation of interleukin 1 (IL-1) receptor expression by IL-1 and fate of internalized ^{125}I-labelled IL-1β in a human large granular lymphocyte cell line. J. Immunol. **137:**3183–3188.
19. **McCarthy, D. O., M. J. Kluger, and A. J. Vander.** 1985. Suppression of food intake during infection: is interleukin-1 involved? Am. J. Clin. Nutr. **42:**1179–1182.
20. **Miossec, P., C. L. Yu, and M. Ziff.** 1984. Lymphocyte chemotactic activity of human interleukin-1. J. Immunol. **133:**2007–2011.
21. **Miyasaka, N., K. Sato, M. Goto, M. Sasano, M. Natsuyama, K. Inoue, and K. Nishioka.** 1988. Augmented interleukin-1 production and HLA-DR expression in the synovium of rheumatoid arthritic patients. Possible involvement in joint destruction. Arthritis Rheum. **31:**480–486.
22. **Mizel, S. B., P. L. Killian, J. C. Lewis, K. A. Paganelli, and R. A. Chizzonite.** 1987. The interleukin 1 receptor. Dynamics of interleukin 1 binding and internalization in T cells and fibroblasts. J. Immunol. **138:**2906–2912.
23. **Movat, H. Z., M. I. Cybulsky, and I. G. Colditz.** 1986. Emigration and accumulation of PMN-leukocytes induced by endotoxin, interleukin 1 and other chemotactic substances. Folia Histochem. Cytobiol. **24:**75–88.
24. **Neta, R., M. B. Stein, J. J. Oppenheim, S. Gillis, and S. D. Douches.** 1987. The *in vivo* effects of interleukin-1. I. Bone marrow cells are induced to cycle after administration of interleukin-1. J. Immunol. **139:**1861–1866.
25. **Otterness, I. G., P. A. Seymour, H. W. Golden, J. A. Reynolds, and G. O. Daumy.** 1988. The effects of continuous administration of murine interleukin-1α in the rat. Physiol. Behav. **43:** 797–804.
26. **Philip, R., and L. B. Epstein.** 1986. Tumor necrosis factor as immunomodulator and mediator of monocyte toxicity induced by itself, γ-interferon and interleukin-1. Nature (London) **323:** 86–89.
27. **Posthelwaite, A. E., L. B. Lachman, C. L. Mainardi, and A. H. Kang.** 1983. Interleukin-1 stimulation of collagenase production by cultured fibroblasts. J. Exp. Med. **157:**801–806.
28. **Powanda, M. C.** 1985. The role of interleukin-1 in homeostasis, p. 535–546. *In* M. J. Kluger, J. J. Oppenheim, and M. C. Powanda (ed.), The physiologic, metabolic and immunologic actions of interleukin-1. Alan R. Liss, Inc., New York.
29. **Powanda, M. C., and W. R. Beisel.** 1982. Hypothesis: leukocyte endogenous mediator/endogenous pyrogen/lymphocyte activating factor modulates the development of nonspecific and specific immunity and affects nutritional status. Am. J. Clin. Nutr. **35:**762–768.
30. **Prystowsky, M. B., M. F. Naujokas, E. Goldwasser, and F. W. Fitch.** 1984. A microassay for colony-stimulating factor based on thymidine incorporation. Am. J. Pathol. **114:**149–156.
31. **Raines, E. W., S. K. Dower, and R. Ross.** 1988. Interleukin-1 mitogenic activity for fibroblasts and smooth muscle cells is due to PDGF-AA. Science **243:**393–396.
32. **Sauder, D. N., N. L. Mounessa, S. I. Katz, C. A. Dinarello, and J. I. Gallin.** 1984. Chemotactic cytokines: the role of leukocytic pyrogen and epidermal cell thymocyte-activating factor in neutrophil chemotaxis. J. Immunol. **132:**828–832.
33. **Schwarz, T., A. Urbanska, F. Gschnait, and T. A. Luger.** 1987. UV-irradiated epidermal cells produce a specific inhibitor of interleukin 1 activity. J. Immunol. **138:**1457–1463.
34. **Seckinger, P., J. W. Lowenthal, K. Williamson, J.-M. Dayer, and H. R. McDonald.** 1987. A urine inhibitor of interleukin 1 activity that blocks ligand binding. J. Immunol. **139:**1546–1549.
35. **Seymour, P. A., D. L. Larson, and R. G. Browne.** 1986. The effect of piroxicam on locomotor activity in rats with adjuvant-induced arthritis. Drug Dev. Res. **7:**165–172.
36. **Shore, A., S. Jaglal, and E. C. Keystone.** 1986. Enhanced interleukin-1 generation by monocytes *in vitro* is temporarily linked to an early event in the onset or exacerbation of rheumatoid arthritis. Clin. Exp. Immunol. **65:**293–302.
37. **Socher, S. H., A. Friedman, and D. Martinez.** 1988. Recombinant human tumor necrosis factor induces reductions in food intake and body weight in mice. J. Exp. Med. **167:**1957–1962.
38. **Stimpson, S. A., F. G. Dalldorf, I. G. Otterness, and J. H. Schwab.** 1988. Exacerbation of arthritis by IL-1 in rat joints previously injured by peptidoglycan-polysaccharide. J. Immunol. **140:**2964–2969.
39. **Tartakovsky, B., A. Finnegan, K. Muegge, D. T. Brody, E. J. Kovacs, M. R. Smith, J. A. Berzofsky, H. A. Young, and S. K. Durum.** 1988. IL-1 is an autocrine growth factor for T-cell clones. J. Immunol. **141:**3863–3867.
40. **Tracey, K. I., H. Wei, K. R. Manogue, Y. Fong, D. G. Hesse, H. T. Nguyen, G. C. Kuo, B. Beutler, R. S. Cotran, A. Cerami, and S. F. Lowry.** 1988. Cachectin/tumor necrosis factor induces cachexia, anemia and inflammation. J. Exp. Med. **167:**1211–1227.
41. **Van der Meer, J. W. M., M. Barzo, S. M. Wolff, and C. A. Dinarello.** 1988. A low dose of recombinant interleukin 1 protects granulocytopenic mice from lethal gram-negative infection. Proc. Natl. Acad. Sci. USA **85:**1620–1623.
42. **Vogel, S. N., S. D. Douches, E. N. Kaufman, and R. Neta.** 1987. Induction of colony stimulating factor in vivo by recombinant interleukin-1α and recombinant tumor necrosis factor α. J. Immunol. **138:**2143–2148.
43. **Wahl, S. M., L. M. Wahl, and J. B. McCarthy.** 1978. Lymphocyte-mediated activation of fibroblast proliferation and collagen production. J. Immunol. **121:**942–946.
44. **Wallach, D., H. Holtmann, H. Engelmann, and Y. Nophar.** 1988. Sensitization and desensitization to lethal effects of tumor necrosis factor and IL-1. J. Immunol. **140:**2994–2999.
45. **Wood, D. D., E. J. Ihrie, C. A. Dinarello, and P. L. Cohen.** 1983. Isolation of an interleukin-1-like factor from human joint effusions. Arthritis Rheum. **26:**975–983.