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Interleukin-1 from P388D$_1$: Effects upon Neutrophils, Plasma Iron, and Fibrinogen in Rats, Mice, and Rabbits (42085)

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Abstract. Partially purified interleukin-1 was prepared from the murine cell line P388D$_1$. This interleukin-1 produced fever in rabbits and the amount required to cause an increase of 1°C was determined. This dose of interleukin-1 produced neutrophilia when injected into rats and rabbits but not in mice, and increased plasma iron and fibrinogen in all three species. Although the mouse was a poor responder to murine interleukin-1 for neutrophilia, it responded unusually well for increasing plasma fibrinogen.

Recent investigations indicated that the monokines known as interleukin-1 (IL-1), endogenous pyrogen (EP), and leukocytic endogenous mediator (LEM) are closely related (1–6). The mouse macrophage cell line P388D$_1$ has been shown to produce IL-1, which is a single polypeptide chain exhibiting microheterogeneity with mol wt of 12,000 to 16,000 and isoelectric points between 5 and 5.4 (8, 9). In addition to its role in lymphocyte responses, IL-1 from P388D$_1$ stimulates collagenase and prostaglandin production by human rheumatoid synovial cells (10). When injected into mice this IL-1 will cause fever (11) and stimulate the synthesis of serum amyloid A (6).

The results presented in this report show that mouse IL-1 shares with LEM the properties of increasing peripheral blood neutrophils, decreasing plasma iron, and promoting the synthesis of fibrinogen. It will produce these effects in rats and rabbits as well as in the mouse, its species of origin.

Materials and Methods. *Animals.* New Zealand white rabbits weighing 3 to 4 kg were purchased locally. Male Holtzman-derived rats, 8 to 10 weeks old, were from our colony. Male C_3H/HeJ mice purchased from Jackson Laboratory (Bar Harbor, ME) were used at 8 to 10 weeks of age.

Preparation of IL-1. The IL-1 was a gift from Eli Lilly and Company (Indianapolis, Ind.). It was prepared from the mouse macrophage cell line, P388D$_1$, by the method of Mizel (8) with an additional step of Sephadex G-25 chromatography. This IL-1 had a mol wt and isoelectric point similar to those described by Mizel (8). When this semipurified preparation was diluted to 10 μg/ml, the rate of incorporation of tritiated thymidine into mouse thymocytes showed a 50% maximal response at a dilution of 1/128 (9). Tests for endotoxin by the Limulus amoebocyte lysate test indicated that this preparation had less than 0.01 ng of endotoxin/ml (12). This amount of endotoxin has been shown not to increase plasma fibrinogen (13), blood neutrophils (14), or to decrease plasma iron (15).

Phorbol myristic acetate was used to stimulate the P388D$_1$ cells and small amounts could still be present in the IL-1 preparation. To check its effects we injected 1 ml/animal of the concentration used in stimulating the cells (1 μg/ml). This amount of phorbol myristic acetate did not effect plasma iron, neutrophils, or fibrinogen in any of the species of animals used in these studies.

Pyrogenic activity. Temperatures were measured rectally in rabbits previously trained for fever determination. Rectal thermometers (YSI 700 series; Yellow Springs Instrument Co., Yellow Springs, Ohio) were taped in place and connected to Datalogger 1000 (Digitec United Systems Corp., Dayton, Ohio). After a stable base line was established, the IL-1 was injected iv, and the temperature was automatically recorded every 5 min.

We determined the pyrogenicity of the IL-1 by plotting the log dose response at four different doses. Only those doses which gave an average temperature elevation of between 0.3 and 0.8°C were used to plot the log dose response curve. The pyrogenic activity was expressed in arbitrary units, where 1 unit was the calculated amount of IL-1 needed to

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produce a mean febrile response of 1°C in rabbits (16).

Other biological activities. IL-1 (1 pyrogenic unit) was injected ip into mice, by the marginal ear vein into rabbits, and iv for measuring neutrophils and ip for assays of plasma iron and fibrinogen in rats. Blood samples were obtained from the marginal ear veins of rabbits and from the heart of anesthetized mice and rats. The blood samples were taken at 6 hr for determining neutrophils and plasma iron levels and at 16 hr for fibrinogen levels in mice. The blood samples in both rabbits and rats were taken at 1 hr for neutrophils, at 8 hr for plasma iron levels, and at 24 hr for fibrinogen levels.

Total leukocyte numbers were counted in a hemacytometer, and the percentage of neutrophils was determined from a 200-cell differential count of a Wright-stained smear. Plasma iron levels were determined in deproteinized samples by atomic absorption spectroscopy on a Model 403 Perkin-Elmer spectrophotometer. Flame was used for rat and rabbit plasma, and the HGA-2000 graphite furnace (Perkin-Elmer Corp., Norwalk, Conn.) was used for mouse plasma. Fibrinogen was assayed in all three species by the heat-turbidity method of Wycoff (13).

Data analysis. The data in the figures are expressed as means ± standard error of the mean. Statistical significance was determined by utilizing the two-tailed unpaired Student *t* test employing a confidence level of 95%.

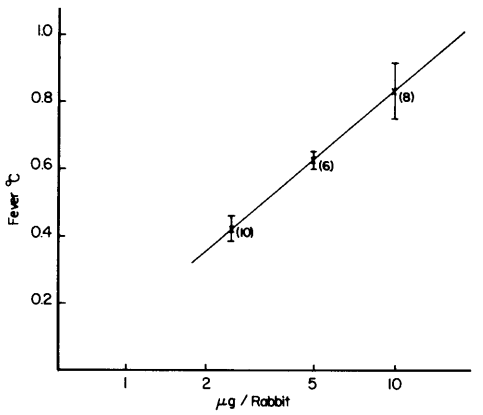

FIG. 1. The pyrogenic effect of injecting various doses of IL-1 prepared from P388D$_1$ cell line into rabbits. The number of trials is shown in parentheses and the standard error is indicated by the bars.

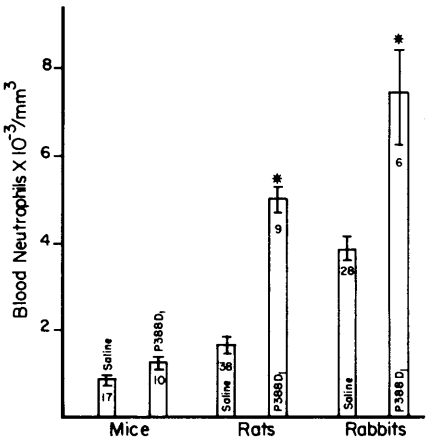

FIG. 2. The effect of murine IL-1 upon peripheral blood neutrophils of mice, rats, and rabbits. The standard error of the mean is shown by brackets, and the number of animals in each group is given below these brackets. Each animal received 1 pyrogenic unit of IL-1, administered ip in mice and iv in rats and rabbits. *Significantly different from the saline control ($P < 0.05$).

Results. The effects of injecting IL-1 prepared from P388D$_1$ upon the body temperature of rabbits are shown in Fig. 1. This log dose response curve indicates that it required approximately 15 μg to produce 1 pyrogenic unit.

Shown in Fig. 2 are the changes in peripheral blood neutrophils when 1 pyrogenic unit of IL-1 was injected per animal into mice, rats, and rabbits. This dose did not cause a significant elevation of neutrophils in mice. The same dose administered to the much larger rats and rabbits produced a two- to threefold increase in neutrophils. Mouse neutrophils could be increased with higher doses (5–10 pyrogenic units) of mouse IL-1. (Data not shown.)

Figure 3 presents the effects of 1 pyrogenic unit of IL-1 upon plasma iron. All three species showed a slight but significant decrease in transport iron with this dose of IL-1. The percentage decrease was similar in all three species ranging from 20 to 29.

Plasma fibrinogen (Fig. 4) was increased by IL-1 prepared from P388D$_1$ in all three species. The percentage increases in fibrinogen were 77, 42, and 33 for mice, rats, and rabbits, respectively.

Discussion. The partially purified IL-1 from P388D$_1$ cells that we used had 12,800 units (50% maximal response) of IL-1/mg. This is

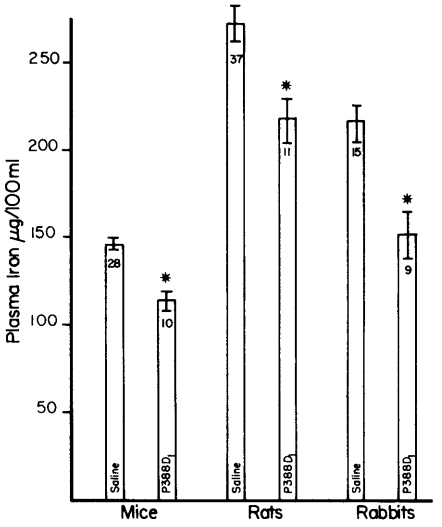

FIG. 3. The effect of murine IL-1 upon plasma iron concentrations of mice, rats, and rabbits. One pyrogenic unit was injected ip into mice and rats and iv into the rabbits. The standard error of the mean is shown by brackets, and the number of animals in each group is shown below these brackets. *Significantly different from the saline control ($P < 0.05$).

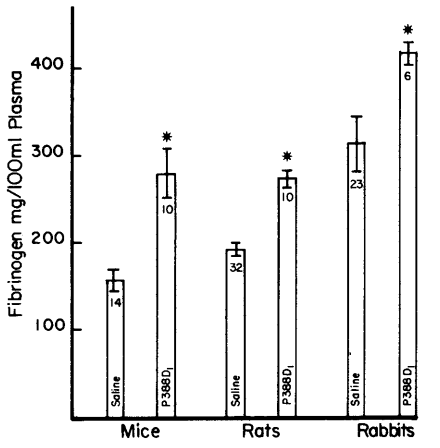

FIG. 4. The effect of murine IL-1 upon plasma fibrinogen levels of mice, rats, and rabbits. One pyrogenic unit was injected ip into mice and rats and iv into rabbits. The standard error is indicated by brackets, and the number of animals in each group is given below these brackets. *Significantly different from the saline control ($P < 0.05$).

5 to 10% of the activity of a homogeneous preparation where Mizel and Mizel (9) set the 50% response at 5 to 10 units of IL-1. It seems possible therefore that a homogeneous preparation may require only 0.5 to 1 μg to produce 1 pyrogenic unit. This is, however, still a fairly high dose in comparison with approximately 50 ng of rabbit or human material required for a pyrogenic response (16, 17).

Mizel *et al.* (18) have prepared antibodies to IL-1 prepared from $P388D_1$ and found six major species of IL-1 by using the antibody as an immunosorbent. It may therefore still be possible that some of these species of IL-1 will show more of a biological response than the others in a particular species of animal. Some indication for this was previously shown for the two biochemically and immunologically distinct EPs from rabbits (19).

Partially purified IL-1 from $P388D_1$ seems to have all of the biological properties of EP and LEM and to be active in mice, rats, and rabbits. The mouse, however, was somewhat unusual in being very unresponsive in the neutrophil assay and quite sensitive for increases in fibrinogen. This would not be the expected response if they were simply due to species specificity. We believe it is more likely due to the unusual responsiveness of mouse fibrinogen to IL-1. Both rabbit (19) and especially rat (unpublished observation) IL-1 are also very active in elevating mouse fibrinogen. We have no satisfactory explanation for the mouse IL-1 having poor neutrophilia-inducing properties in the mouse since the mouse does show a good response to rabbit IL-1 (19, 20).

The results from this report plus previously published data (6, 11) suggest that mouse IL-1 has biological activities similar to those found for EP or LEM from rabbit or human sources (1, 2, 19).

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