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Rate of Clearance of Interleukin-1 from the Blood of Normal and Nephrectomized Rats (42160)

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Abstract. Interleukin-1 (IL-1), a macrophage product, appears to be responsible for a wide variety of changes in animals during the early stages of infection and inflammation. However, the fate of injected IL-1 has not been established. The major pathway of removal from the circulation may be through the kidney, since IL-1 can be found in urine. The IL-1 for these studies was prepared from peritoneal macrophages of rats and rabbits. The clearance rate of IL-1 was determined by measuring the activity, for increasing plasma fibrinogen or releasing bone marrow neutrophils, which remained in the blood at various times after an iv injection. Both rat and rabbit IL-1 were removed rapidly from the blood of rats. The results indicate that less than 10% of the IL-1 was cleared by the kidney. So that rats with their kidneys removed showed only a slight decrease in the clearance rate of IL-1. This suggests that excretion through the kidney does not represent the major mechanism of IL-1 disposal.

Interleukin-1 (IL-1) is a product of macrophages which mediates many different host responses during inflammation or infection (1, 2). This family of proteins has also been called endogenous pyrogen and leukocytic endogenous mediator. The fate of these molecules following intravenous injection has not been clearly established. There is some evidence that IL-1 may be excreted through the kidney since it has been shown to be present in urine (3–5). Other studies suggest that IL-1 is cleared rapidly from the circulation by the liver (6). Rabbit IL-1 with a molecular weight of about 14,000 and an isoelectric point at pH 7.3 (7) was shown to have a half-life of 6–10 min in the blood following an iv injection in rats (8). It would be useful to check the rate of removal of rat IL-1 since the rapid rate shown by rabbit IL-1 could be due to the injection of a protein from a different species.

In the present studies we prepared IL-1 from rat macrophages and found that it was cleared even more rapidly than material prepared from rabbit macrophages. The rate of clearance of IL-1 in nephrectomized rats was slightly decreased from that found in sham-operated controls. This suggests that the amount of IL-1 normally being removed from the circulation by the kidney must be very low.

Materials and Methods. *Animals.* New Zealand white rabbits weighing 3–4 kg were purchased locally. Male, Holtzman-derived rats, 10–12 weeks old, were from our colony.

Nephrectomy. A 20- to 25-mm incision was made in both flanks and the kidney was delivered by the aid of a finger. The adrenal was detached, the pedicle of the kidney ligated and excised. The sham-operated animals were anesthetized and subjected to the same procedure except for the ligation and removal of the kidney. The rats were kept under anesthesia and used for clearance studies within 1 h of the operation.

Preparation of IL-1. The method used for the preparation and partial purification of rabbit IL-1 has been previously described (8). Large retired breeder male rats were infused ip with 50–100 ml of 0.2% shellfish glycogen in saline for the preparation of rat IL-1. Fifteen hours later 3×10^9 heat-killed *Staphylococcus aureus* in 0.15 *M* saline were injected. One hour after this stimulation the peritoneal cells were removed, centrifuged, washed, and suspended in 0.15 *M* NaCl at a concentration of 1×10^8 cells/ml. This suspension was incubated at 37°C for 2 hr, and the supernatant containing IL-1 was separated by centrifugation at 800*g* for 10 min.

Measurement of biological activities. Heart blood, collected with a heparinized syringe

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from anesthetized rats, was used for total and differential leukocyte counts. Total leukocyte numbers were counted in a hemocytometer, and the percentage of neutrophils was determined from a 200-cell differential count of a Wrights' stained smear. Individual rats were used only once in the time-course experiments. Fibrinogen was determined by the heat turbidity method of Wycoff (9).

Determining the rate of IL-1 disappearance from blood. The rats receiving partially purified rabbit IL-1 were given 5 pyrogenic units/rat. Those injected with rat IL-1 were given an iv injection of 1 ml of the crude supernatant. At varying intervals after these injections, heart blood was taken from anesthetized rats and assayed for the amount of IL-1 activity remaining in circulation. The heparinized plasma from each rat was separated by centrifugation and 1 ml/rat was injected iv into two or three normal rats. Peripheral blood neutrophils were determined at 1 hr and fibrinogen 24 hr after the plasma injections.

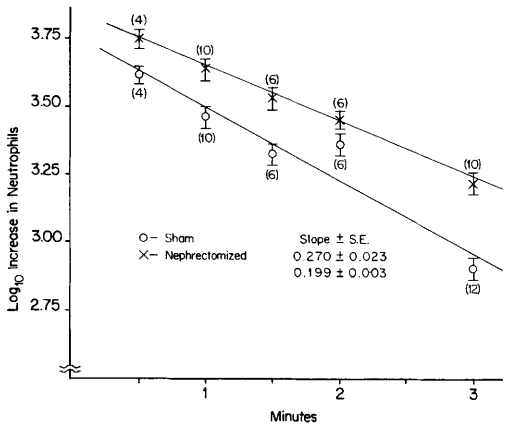

FIG. 1. Rate of disappearance from the plasma of rat IL-1 activity as measured by neutrophil release from bone marrow. Each plasma donor both nephrectomized X and sham-operated 0 had received an iv injection of IL-1 prepared from rat peritoneal macrophages. The plasma from each plasma donor rat was injected into two to three normal rats and circulating neutrophils were determined 1 hr later. The number of rats is shown in parentheses and the standard error by brackets. The number of neutrophils are plotted as Log_{10} and represent the increase above normal values expressed as number/mm^3.

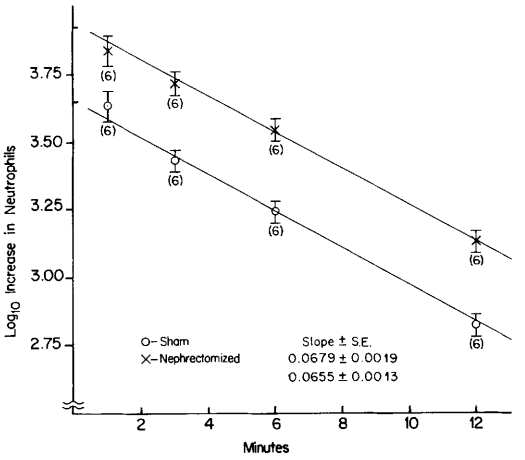

FIG. 2. Rate of disappearance from plasma of rabbit IL-1 as measured by neutrophil release from bone marrow. Each plasma donor both nephrectomized X and sham-operated 0 received an iv injection of 5 pyrogenic units of partially purified rabbit IL-1. The plasma from each donor rat was injected into two to three normal rats and circulating neutrophils were determined 1 hr later. The number of rats is shown in parentheses and the standard error by brackets. The neutrophil values are increases above normal expressed as number/mm^3.

Data analysis. The decrease of IL-1 activity in plasma at various times after injection were plotted as linear regressions. Variance about the slope estimates from the regression of these values on time were computed as described by Natrella (10).

Results. The rate of disappearance of the activity, which increased neutrophils, from the blood in nephrectomized or sham-operated rats when rat IL-1 was injected is shown in Fig. 1. There was a significantly ($P < 0.001$) slower rate of removal in the rats without kidneys. The rate of clearance in both groups after receiving rat IL-1 was rapid with a $T\frac{1}{2}$ of less than 2 min. This very rapid rate made it difficult to draw the blood samples at the proper intervals so the other experiments were done with rabbit IL-1.

Figure 2 shows the rate of disappearance of rabbit IL-1 activity from the blood of rats. The rate of clearance of activity was not significantly different in the sham and nephrectomized rats. The rats without kidneys cleared the rabbit IL-1 somewhat slower, but

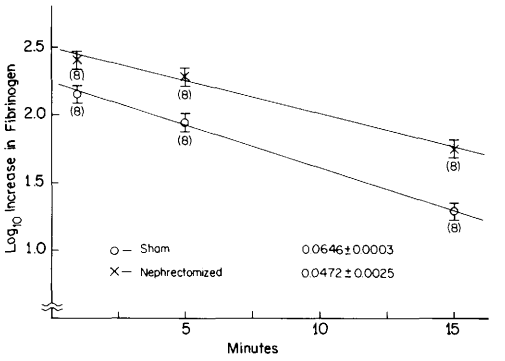

FIG. 3. Rate of disappearance from the plasma of rabbit IL-1 as measured by plasma fibrogen concentration. Each plasma donor both nephrectomized X and sham-operated 0 received an iv injection of 5 pyrogenic units of partially purified rabbit IL-1. The plasma from each donor rat was injected into two to three normal rats and fibrinogen determined 24 hr later by the heat turbidity method (9). The number of rats is shown in parentheses and the standard error by brackets. The fibrinogen values are increases above normal expressed as mg/100 ml of plasma.

both groups removed the rabbit IL-1 at a slower rate than they cleared rat IL-1.

Injection of 1 ml of plasma, from a nephrectomized rat that 1 min previously had received an iv injection of 5 pyrogenic units of rabbit IL-1, into normal rats increased fibrinogen concentration 248 ± 44 mg/100 ml plasma (Fig. 3). Fifteen minutes after the IL-1 injection, 1 ml of the nephrectomized donor plasma increased fibrinogen 46 ± 19 mg/100 ml plasma. Once again the rate of clearance was significantly ($P < 0.001$) slower in the nephrectomized as compared to the sham-operated rats.

Discussion. Interleukin-1 prepared from rat macrophages was cleared from the rats blood faster than rabbit IL-1. The rabbit IL-1 has a mol wt of about 14,000 and an isoelectric point at pH 7.3 (7), while the rat IL-1 has a higher mol wt and isoelectric points between pH 4 and 5 (data in preparation).

The similarities between the rates of removal of the activities for releasing marrow neutrophils (Fig. 2) and increasing fibrinogen (Fig. 3) provide further evidence of their mediation by the same protein (1, 2). There was a slight but significant decrease in IL-1 removal in two of the three experiments using nephrectomized rats. This would suggest that only a small portion of the total IL-1 activity was excreted through the kidney. If this was the major route of removal the $T\frac{1}{2}$ would be expected to increase several fold.

Recent studies have shown that human IL-1 is degraded to a 4200 mol wt peptide which will induce fever, cause proteolysis of muscle, and stimulate thymocyte proliferation (10). This peptide was shown in the plasma of febrile patients (10) and may be related to the small peptides found in human urine (5). It is not known if a similar degradation to an active peptide occurs with rat or rabbit IL-1. It is also presently unknown whether the 4200 mol wt peptide will release marrow neutrophils or increase acute phase protein synthesis. It is therefore still possible that IL-1 is degraded to a low mol wt product which remains in circulation but is not active in releasing marrow neutrophils or increasing plasma fibrinogen. This possibility seems rather remote, since most previous studies have shown a close relationship between the various activities of IL-1 (1, 2).

The slight decrease in the rate of removal of IL-1 when the kidneys are removed is probably realistic since there is substantial evidence that some IL-1 reaches the urine (3–5). Interleukin-1 apparently has several different sites of action and it might be expected to bind briefly in order to effect these actions (1). One logical site would be the liver (6), but it was not possible to verify a role for liver with the techniques used in these experiments. When IL-1 is being cleaved and degraded some of the activity would undoubtedly be lost (10). It may not therefore be possible to find one fate or one site of accumulation of IL-1.

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