Mechanism of Eosinophilia
IX. Induction of Eosinophilia in Rats by Certain Forms of Dextran[1] (36532)

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Experimental investigation of eosinophilia has been handicapped by lack of reproducible methods by which the phenomenon can be provoked. Although parasitic infestation is a reliable stimulus, an active infestation has limitations for many types of study, because of the prolonged and fluctuating eosinophilia which ensues. Previous work in this laboratory has made use of a model in which muscle stage *Trichinella* larvae, living or dead, are injected (1). Because of their size the larvae are arrested in the pulmonary vasculature where they cause an acute inflammatory response, and in which they disintegrate. The method is useful in that it involves a single timed challenge and evokes a uniform eosinophil response. We have employed this procedure to examine eosinophilia as an immunological phenomenon (2–4) and to study the kinetics of the response (5, 6). The latter work shows clearly that the rise in blood level is related to an increased rate of production in bone marrow.

We report here the finding that a single injection of dextran in the form of beads will stimulate eosinophil production in a manner resembling that which results from injection of the parasitic larvae.

Materials and Methods. Animals. Two strains of rats, known to be free of macroparasites, were employed; A Wistar outbred strain (M. R. C. Radiobiological Establishment, Harwell) and an inbred AGUS strain (M. R. C. Laboratory Animal Centre, Carshalton, Surrey). These exhibited comparable patterns of eosinophil response, although the resting level was slightly higher with the AGUS animals. Results refer to experiments using the Wistar strain unless stated otherwise.

Dextrans. Ten milligrams of Sephadex® beads (Pharmacia, G. B.) which are composed of multiply cross-linked dextrans (and no other constituent) were suspended in about 25 ml saline and allowed to stand for 48 hr at 4°. Owing to their hydrophilic property they swell during this period. For counting, a drop of well-agitated suspension was examined in a hemocytometer chamber. The concentration was adjusted to 5×10^4 beads/ml by the addition of further volumes of saline. Table I shows the range of diameters of four grades of Sephadex tested, as measured microscopically with a calibrated eyepiece graticule at ×160 magnification. It will be seen that three of these grades were similar in size, whereas the "G 25 superfine" was smaller.

Rats receiving more than 7×10^4 beads iv showed signs of respiratory distress, whereas doses of 5×10^4 or less were tolerated without apparent difficulties; consequently all tests employed 5×10^4 beads.

Beads were fragmented by grinding in a tissue homogenizer or by sonic disruption. For the latter, 10 ml of suspension was subjected to ultrasound at 4° using a 100-W ultrasonic disintegrator (M. S. E.) at maximum output for a total of 40 sec. Microscopic examination showed irregular small fragments after sonic disruption, while mechanical grinding resulted in slightly larger particles.

Technical grades of dextrans (Pharmacia, G. B.) were dissolved in distilled water at a concentration of 50 mg/ml and injected iv in quantities of 30 mg/100 g body wt.

The method employed in counting eosino-

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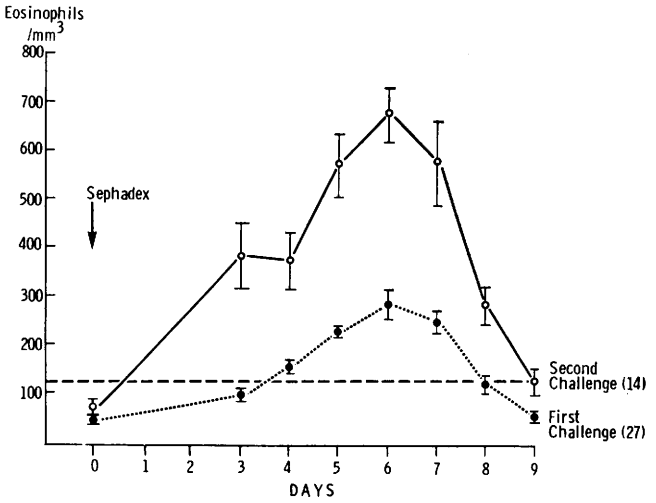

FIG. 1. Peripheral blood eosinophil counts after iv injection of 5 × 10⁷ Sephadex beads. In this and Fig. 2 each point represents the mean of animals counted at a particular time. The range at each point describes ± 1 SE. The horizontal broken line represents the mean + 2 SD of resting counts and is taken to be the upper limit of normal. Numbers of animals in each experiment are given in parentheses.

phils has been described previously (1). Lung tissue was fixed in Bouin's solution and sectioned at 4 μ thickness; sections were stained with hematoxylin and eosin, and with periodic acid–Schiff reagents. Statistical comparisons were performed using Student's t test as described before (1).

Results. Eosinophil response to first and second injection of Sephadex. Figure 1 shows the blood eosinophil levels after two injections of "G 200 superfine" beads 4 weeks apart. These responses are similar to those obtained with *Trichinella* larvae in that the rise begins after the 2nd day, reaches a peak on the 6th or 7th day, and returns to within the normal range by the 10th day; furthermore, a second challenge 4 weeks later evokes an augmented response.

The mean eosinophil responses with the four grades of Sephadex tested are shown in Table I. The effect of the "G 25 superfine" appeared to be less than that of the other three although it was not statistically significant in the small numbers employed. "G 200 superfine" was used in all subsequent tests.

Effect on other circulating leukocytes. Differential leukocyte counts were carried out on five rats during 10 days after injection of Sephadex beads. Whereas the eosinophil counts rose at least fivefold, the neutrophil count did not exceed twice the resting level, and mononuclear cell numbers remained within the normal range.

Fragmentation of beads before injection. It had previously been found that homogenization of *Trichinella* larvae into fragments small enough to traverse the pulmonary circulation would abolish the eosinophil response. An attempt was made, therefore, to fragment Sephadex beads, so that the same phenomenon could be tested for. Adequate fragmentation proved technically difficult; nevertheless such treatment did diminish significantly their capacity to stimulate eosinophilia ($p < .05$ after homogenization, and $< .005$ after treatment with ultrasound).

Prevention of eosinophil response by immunosuppressive agents. In view of the previous finding (3) that single doses of certain immunosuppressive agents given at appropriate times would abolish the eosinophil response to *Trichinella*, comparable tests were done with Sephadex in AGUS rats. Figure 2 shows that prednisolone given 6 hr before Sephadex prevents the eosinophil response ($p < .001$). Methotrexate given 24 hr after dextran was also effective ($p < .025$). These results resemble those obtained when *Trichinella* larvae were used as the stimulus.

Histologic reaction in the lung after injec-

TABLE I. Eosinophil Responses Induced by Various Grades of Sephadex.

Grades of Sephadex	Particle sizes of Sephadex beads (μ)			Number of rats	Peripheral blood eosinophils/mm^3 (mean of the mean responses for days 4–8 ± 1 SE)
	Mean diameter ± 1 SD	Range of diameters	Number of beads measured		
G200 Superfine	66.8 ± 22.9	23 – 109	44	4	200 ± 24
G10	67.4 ± 19.4	41.5 – 106	26	4	178 ± 11
G25 Fine	55.2 ± 25.3	12 – 103	25	4	174 ± 37
G25 Superfine	29.8 ± 14.9	12 – 59	27	4	118 ± 28

tion of Sephadex. Sections of rat lungs examined at intervals after injection of Sephadex disclosed a sequence of inflammatory changes similar to those evoked by *Trichinella* larvae (7). At 3 and 6 hr there were small collections of neutrophils and mononuclears about the impacted beads. By 12 hr the neutrophil element was no longer evident and the lesion consisted of large mononuclear cells with eosinophils admixed. By 24 hr (Fig. 3) this had developed further with mononuclear cells tightly packed around each bead. Multinucleate giant cells were seen. Eosinophils were conspicuous at the periphery of this cellular reaction.

Effect of dextrans in colloidal solution. Three samples with average molecular weights of 8×10^4, 1.6×10^5, and 2×10^6 were tested in AGUS rats. The first two had no effect on circulating eosinophil levels. However, the polymer with the highest molecular weight evoked a variable response which was significantly elevated overall ($p < .05$) but which did not exceed the upper limit of normal in four of nine rats tested. It should be pointed out that the dose of dextran used was about 100 times by weight that of Sephadex beads and that when comparable quantities were administered there was no longer a significant response to soluble dextran.

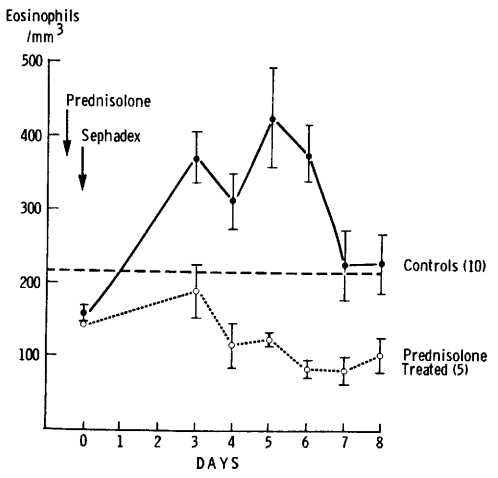

FIG. 2. Prevention of eosinophil leukocytosis after injection of Sephadex. Prednisolone was given 6 hr before Sephadex.

All three preparations of dextran caused the well-known effect observed in rats given these substances intravenously—swelling of the muzzle and paws accompanied by transient respiratory difficulty (8). Injection of Evans blue dye at this time caused intense blueing of the areas of cutaneous swelling. This reaction appeared no more severe with the preparation which provoked eosinophilia than with the other two. Histologic examination of subcutaneous tissue of muzzle, hindpaw and tail, as well as of gut and lung from rats injected 24 hr previously with dextrans, showed no gross variation from normal, nor could any differences be observed between the recipients of dextrans of varying molecular weights.

Discussion. This work is of interest because it shows that a simple substance of known chemical structure will regularly stimulate increased eosinophil production when injected in the form of beads large enough to be trapped in the lungs. Attempts to provoke eosinophilia experimentally with antigens such as ovalbumin or plasma protein have seldom been productive, and then only after several challenges. Clinical experience corrob-

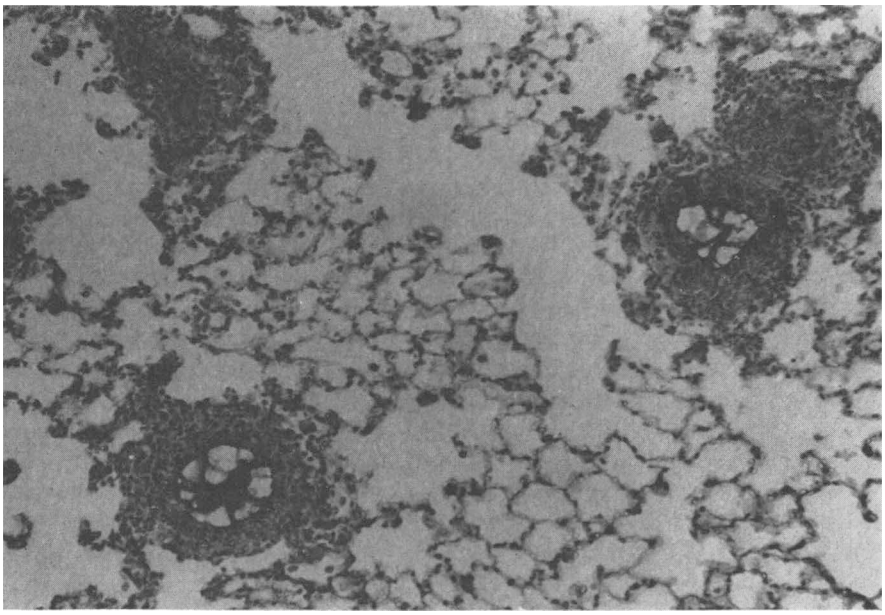

FIG. 3. Inflammatory response to Sephadex in the lung, 24 hr after injection. Mononuclear cells are packed tightly about the beads, but eosinophil leukocytes are conspicuous in the periphery of the lesions. Giant cells are seen in some of the lesions. PAS stain. Magnification ×140.

orates this in that many drugs which can cause eosinophilia do so irregularly and only after repeated administration.

We believe that the effect of Sephadex beads in eliciting eosinophilia is related in part to their size, necessitating that the antigen is trapped in the lungs instead of being carried to the reticuloendothelial system. Evidence in our other investigations on the mechanism of eosinophilia points to a dependence on the character of local tissue reaction to the foreign substance (1). The inflammatory response in the lung to dextran beads closely resembles that which develops after injection of *Trichinella* larvae. It should be mentioned here that inert particles of similar size, polystyrene beads, cause little inflammatory response where they lodge in the lungs, and no detectable rise in circulating eosinophils. Fragmentation of Sephadex beads reduced the eosinophil response as did homogenization of *Trichinella* larvae. It seems possible that the variable eosinophil response to the large molecular weight dextran could result from inflammation in some tissues. Mayerson *et al.* (9) have found regional differences in capillary permeability to dextrans depending on their molecular weights. It is likely that the anaphylactoid reaction resulted in further leakage of dextran from capillaries. Perhaps relevant in this connection is the finding that footpad injections of dextrans with molecular weights of 5–40 $\times$ 10^6 produced diffuse eosinophil infiltrations in the regional popliteal lymph nodes of rabbits, whereas the same quantities of dextrans with molecular weights of 6–9 $\times$ 10^4 failed to have that effect (10).

The antigenicity of dextrans has been studied in several hosts. In man immediate hypersensitivity reactions and precipitating antibody are known to be induced by dextrans of high molecular weight (11). Precipitating antibodies to dextran have not thus far been demonstrated in rats (12). Because of its simple chemical structure, dextran appears to be recognized as a single antigenic moiety by the guinea pig (13), and in man it elicits the formation of unusually homogenous antibody (14). This contrasts with the extremely complex antigenic nature of parasitic material which elicits a wide-range of antibodies.

The simple method described here for pro-

voking a predictable eosinophil leukocytosis by a single challenge with Sephadex beads may prove useful in further studies on the mechanism of eosinophilia.

Summary. Intravenous injection of dextran in the form of beads regularly induces eosinophil leukocytosis in rats. The response has characteristics of an immunological reaction.

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