Isolation of the Leukocyte Chemotactic Factors in the Extract of Adult *Metastrongylus apri*.

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Isolation of the chemotactic components in the extract from adult *Metastrongylus apri* (*M. apri*) possessing chemotactic activity for eosinophils, neutrophils and macrophages of guinea pigs, was attempted. The components were assumed to be in a fraction separated by salting-out with 33 to 50% saturation of ammonium sulphate. The majority of the activity was contained in one of two protein peaks resolved by gel-filtration with Sephadex G-100. The active peak, neighbouring that of bovine γ -globulin, was divided into several peaks showing leukotactic activities with isoelectric focusing. Component a focused at pH 4.8 ± 0.2 was specific for only macrophages, while c focused at pH 7.4 ± 0.2 for both macrophages and eosinophils. Component b focused at pH 6.7 ± 0.2 was active for neutrophils, macrophages and eosinophils, and component d found at pH 5.2 ± 0.2 was specific only for neutrophils. A chemotactic activity for eosinophils was found in a predominant peak focused at pH 5.8 to 7.8. In these fractions, the highest activity was shown by component e, of a peak at pH 7.2 ± 0.2 .—**Key words:** Leukocyte chemotaxis, *Metastrongylus apri*.

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As previously reported (3), the extract from adult *Metastrongylus apri* (*M. apri*) showed chemotactic activity for eosinophils, neutrophils and macrophages of normal guinea pigs, *in vivo* and *in vitro*. However, it remains unknown whether such activity depends on one or more factors being specific for each species of leukocytes. In this study an attempt was made to isolate active proteins or proteinaceous factors (3) from extract of *M. apri* by salting-out, gel-filtration and isoelectric focusing.

MATERIALS AND METHODS

Extract of adult *M. apri*: A *M. apri* extract (protein 4.1 mg/ml) was prepared from adult worms according to the methods described previously (3).

Ammonium sulphate fractionation: Solid ammonium sulphate was added little by little to the *M. apri* extract containing 20.5 mg of

protein until 33% saturation. After storing at 4°C for 12 hr, the precipitate was removed by centrifugation at 10,000 $\times g$ for 15 min at 4°C. To the supernatant ammonium sulphate was added until either 50 or 80% saturation. All the precipitated fractions were insoluble in phosphate buffered saline, pH 7.4 (PBS), and the supernatants fractions at 33%, 50% and 80% saturations were dialyzed against PBS for 48 hr at 4°C, and then subjected to chemotactic assay.

Gel filtration on Sephadex G-100: Three ml of the *M. apri* extract containing 12.3 mg protein were applied to a Sephadex G-100 column (2.5 $\times$ 40 cm) equilibrated with 0.01 M PBS. Elution was made with the same buffer at a flow rate of 9 ml/hr and 50 fractions of about 4 ml effluent were collected. For determining molecular weight, bovine γ -globulin, ovalbumin, cytochrome C and vitamin B₁₂ were gel-filtrated by the

same manner.

Isoelectric focusing: The lyophilized preparation of the first protein peak of the gel filtration containing 5.1 mg of protein was dissolved in 3 ml of distilled water and dialyzed against 1% glycine. The solution was subjected to isoelectric focusing with a preparative flat bed electrofocusing in a granulated gel (LKB, 2117, 5% carrier ampholyte, pH range 3.5 to 10) for 48 hr at 10°C, and thirty fractions were obtained from the gel bed with a focusing grid according to the method described by Rodal (1). Three ml of each effluent fraction was collected for determination of the protein concentration and *in vitro* chemotactic activity.

***In vitro* chemotactic activity:** After adjusting protein concentrations to 50 or 25 µg/ml, the extract and its fractions were examined for chemotactic activities for guinea pig eosinophils, neutrophils or macrophages, using the modified Boyden chamber, of which samples were prepared as described previously (3). The chemotactic activity was represented by the mean of emigrated cell counts, and the total activity was calculated as follows: Total activity = chemotactic activity × (total protein (µg)/50 µg)

Protein and carbohydrate determination: Protein content was determined using the Folin phenol reagent and an optical density at 280 nm. Carbohydrate content was detected by anthrone reaction.

RESULTS

Activity of the salting-out fraction with ammonium sulphate: As already mentioned, the salting-out fractions of *M. apri* extract were insoluble in PBS, and thus the supernatants were examined for leukocyte chemotaxis, as shown in Table 1. Chemotactic activity of the supernatant of 33%-fraction was remarkable for macrophages and neutrophils, being much higher than that of the original extract. Also a relatively high activity of this fraction was shown for eosino-

Table 1. Chemotactic activities of supernatant of salting-out fractions from *M. apri* extract.

Materials ^{a)}	Activity for ^{b)}		
	Eosino- phils	Neutro- phils	Macro- phages
Original extract	34	296	84
Supernatant of (NH ₄) ₂ SO ₄ saturation at:			
33%	16	396	127
50%	10	58	42
80%	8	44	21

a) Protein: 50 µg/ml.

b) Mean of emigrated cell counts.

phils, the supernatants of 50%-and 80%-fraction, however, activity was lower.

Activity of the gel-filtrated fractions: The effluent through the Sephadex G-100 gel showed two protein peaks, as shown in Fig. 1. The first near the bovine γ-globulin peak and the second being close to that of vitamin B₁₂. Seventy two per cent of the total protein in the *M. apri* extract was recovered from the first peak and 22% from the second. The first peak contained minimal carbohydrates, while the second peak had a much higher carbohydrate content.

The chemotactic activities of the two peaks are shown in Fig. 2. Fraction Nos. 14, 15 and 17 exhibited for macrophages, whereas Nos. 15 or 17, and No. 12 showed the highest chemotactic activities for neutrophils and eosinophils respectively. These activities of these fractions were higher than those of the original *M. apri* extract. The highest activities for neutrophils and macrophages were recovered with the same effluent fractions except for No. 14, but that for eosinophils was with a specific one, suggesting the existence of specific factors for each leukocyte species. All these active fractions were contained in the first peak and the fractions in the second peak had less activity. Thus, the first peak contained total activity more than 90% and 60% of original *M. apri* extract for eosinophils or macrophages, and neutrophils,

Fig. 1. Elution profile of the extract from adult *M. apri* on Sephadex G-100 gel-filtration.

1) Bovine γ -globulin. 2) Ovalbumin. 3) Cytochrome C. 4) Vitamin B₁₂.

Fig. 2. Chemotactic activities of gel-filtrated fractions of *M. apri* extract.

1) Mean of emigrated cells, protein 50 μ g/ml.

Fig. 3. Total activities of gel-filtrated fractions of *M. apri* extract.

1) Total activity = chemotactic activity $\times$ (total protein (μ g)/50 μ g).

Fig. 4. Chemotactic activities of isoelectric focusing fractions of the first peak on Sephadex G-100 gel-filtration.

1) See the legends to Table 1. protein 25 $\mu\text{g/ml}$

respectively, as shown in Fig. 3.

Activities of the fractions prepared by isoelectric focusing: The first peak of the gel-filtration containing most activity of the original extract was subjected to fractionation by isoelectric focusing. As shown in Fig. 4, a high activity for macrophages was found in 3 fractions focused at $\text{pH } 4.8 \pm 0.2$ (component a), $\text{pH } 6.7 \pm 0.2$ (component b) and $\text{pH } 7.4 \pm 0.2$ (component c). Components a and c were specific for macrophages and for both macrophages and eosinophils respectively, while component b was active for macrophages, neutrophils and eosinophils. The activity for neutrophils was found in the b component as well as component d focused at $\text{pH } 5.2 \pm 0.2$. The activity for eosinophils was found in all fractions focused at $\text{pH } 5.8$ to

7.8, peaking at a fraction e focused at $\text{pH } 7.2 \pm 0.2$. However, less or no activities were recovered in other fractions.

DISCUSSION

The extract from adult *M. apri* had chemotactic activities for eosinophils, neutrophils and macrophages (3). Most activities were recovered in a fraction precipitating at 33 to 50% saturation of ammonium sulphate (Table 1). The molecular weight of the active protein was estimated to be approximate to that of bovine γ -globulin (16,000 daltons), by gel-filtration with Sephadex G-100 (Fig. 1 and 2). The active components differed from a value of 30,000 daltons and less described with the chemotactic factors of *Ascaris* by Tanaka *et al.* (5). By the isoelectric focusing of the active peak, five different components a, b, c, d and e, were obtained (Fig. 4). Components a and d were specific for macrophages and neutrophils, respectively and e was specific for eosinophils. Components b and c were specific for both eosinophils and macrophages, and component b also for neutrophils. These indicate that the chemotactic activities of adult *M. apri* extract depend on at least these five protein components having different specificities, though many other components have a slight activity for eosinophils.

Also we found that a protein-like substance of the third-stage larvae of *M. apri* had a high chemotactic activity for eosinophils but not for neutrophils and macrophages (4). The third-stage larvae may abound in component e but be lacking in other components.

On the other hand, the chemotactic factors of *Taenia taeniaformis* (1) showed that two predominant peaks focused at $\text{pH } 6.8$ to 7.4 and 8.2 to 8.6 , were highly chemotactic for both eosinophils and neutrophils. Also by Tanaka *et al.* (5), the isoelectric point of chemotactic factors for eosinophils and neutrophils from *Ascaris* were $\text{pH } 8.5$ and $\text{pH } 5.2$ or 7.6 . These data of *T. taeniaformis* and

Ascaris were not similar to those of *M. apri* extract in our studies, suggesting that chemotactic components are diverse with regard to worm species.

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要 約

豚肺虫成虫抽出液の白血球遊走因子の分離について：佐々木 脩・勝野正則¹⁾（秋田県立農業短期大学家畜衛生学教室，²⁾東北大学農学部家畜衛生学教室）——正常モルモットの好酸球，好中球，マクロファージに対する遊走因子を含む成熟豚肺虫の抽出液を硫酸塩析，Sephadex G-100 ゲル濾過，等電点分画法により分画した。遊走因子は 33～50% 硫酸飽和で沈降する蛋白部分にあるものと推察された。Sephadex G-100 によるゲル濾過では，2 峰性の蛋白質ピークのうち第 1 峰に遊走因子の大部分が含まれ，その分子量は約16万と推察された。この第 1 峰を等電点分画法により分画したところマクロファージ遊走因子は，pH 4.8±0.2，6.7±0.2 と 7.4±0.2 の画分に，好中球遊走因子は pH5.2±0.2 と 6.7±0.2 の画分に高い活性が認められた。しかし好酸球に対する活性は pH 7.2±0.2 をピークとして pH 5.8～7.8 の画分に幅広く分布した。これらのことから，豚肺虫成虫抽出液中には各種白血球に特異性の高い遊走因子と，それらに共通に作用する因子があることが示唆された。