

Enhanced Release of an Alveolar Macrophage-Derived Chemoattractant for Fibroblasts in Rats after Asbestos Inhalation

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ABSTRACT. Our studies indicate the effects of *in vivo* asbestos exposure on the ability of alveolar macrophages (AM) to elaborate a chemoattractant for fibroblast using a rat model of asbestos inhalation. Two groups of rats were exposed by intermittent inhalation (6 hr/day for 5 days/week over a total period of 4 weeks) to either amphibole (crocidolite) or serpentine (chrysotile) asbestos. A group of control rats were sham-exposed to clean air only. The animals were sacrificed 2–5 months after the cessation of exposure. The AM were obtained from the 3 exposure groups in 2 different rat strains by the bronchoalveolar lavage and the cultured in RPMI-1640 medium for 24–96 hr at 37°C. The supernatants from cultured AM were tested for chemotactic activity towards fetal rat skin fibroblasts in a chemotactic assay using 8 μ m pore-size filters. The culture supernatants of AM obtained from crocidolite-exposed rats exhibited a significantly greater chemotactic activity towards rat fibroblasts than similar culture supernatants from sham-exposed control animals ($p < 0.01$) in both rat strains. Significant chemotactic activity was observed after chrysotile exposure ($p < 0.05$) in ACI rats but not in Fischer-344 rats. Maximal chemoattractant release from AM was noted after 48 hr in culture. Preliminary characterization of the chemoattractant has shown that it is a thermolabile and trypsin sensitive factor whose activity was partially reduced after dialysis. Since AM accumulate at sites of intrapulmonary asbestos deposition, these findings may have relevance to the pathologic accumulation of interstitial lung fibroblasts which occurs during asbestos-mediated lung injury.—**KEY WORDS:** alveolar macrophage, amphibole asbestos, chemoattractant, fibroblast, serpentine asbestos.

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Asbestosis is a disease which is characterized by diffuse interstitial pulmonary fibrosis following the inhalation of asbestos fibers. Several studies demonstrated increased numbers of fibroblasts in the pulmonary interstitium as a consequence of chronic asbestos-mediated lung injury [5, 6, 37]. Alveolar macrophages (AM), which are also a prominent component at the sites of intrapulmonary asbestos deposition, have been implicated in the pathogenesis of asbestosis [7, 19]. We previously demonstrated that asbestos inhalation can induce dramatic alterations in the AM subpopulation. These were characterized by an increased proportion of multinucleated and higher-density AM [18], and enhanced Ia antigen expression of AM [11, 18, 20]. These findings have indicated that some of these newly recruited macrophages were functionally activated [18]. Kagan *et al.* [21] have demonstrated the enhanced release of chemoattractants for rat AM from cultured AM after asbestos inhalation. AM are known to be able to secrete many kinds of inflammatory mediators in response to a variety of stimuli [3]. Pulmonary fibrosis, a progressive, debilitating disease is characterized by the deposition of an increased amount of collagen which results in the accumulation of fibroblasts in the lung. It is, therefore possible that fibroblasts may also be recruited to sites of asbestos-mediated pulmonary injury as AM. In this regard, it is pertinent that stimulated AM release the glycoprotein, fibronectin, which is a potent fibroblast chemoattractant [33]. It is conceivable that alveolar macrophages may similarly elaborate chemoattractants for fibroblasts at sites of asbestos-mediated

lung injury. The present work was undertaken to confirm this hypothesis.

MATERIALS AND METHODS

Experimental animals and exposure protocols: The study entailed a comparison of three matched groups of adult male, inbred ACI and Fischer-344 rats. The exposures were performed under contract at the University of Vermont as described previously [18]. Briefly, in both strains, 2 groups of animals were exposed in 8.0 m³-capacity stainless-steel and glass inhalational chambers by intermittent inhalation (6 hr/day for 5 days/week over a total period of 4 weeks) to either a time-weighted average concentration of 10.52 mg/m³ of amphibole (crocidolite) or a time-weighted average concentration of 10.23 mg/m³ of serpentine (chrysotile) asbestos. A control group of each strain was similarly exposed to clean air only. Similar numbers of rats from the three exposure groups were sacrificed at similar times, ranging from 2 to 5 months after the cessation of exposure. Histologically, mild interstitial pulmonary fibrosis was observed in the lungs from asbestos-exposed group of rats.

Bronchoalveolar lavage: The rats from each groups were sacrificed simultaneously 2–5 months after the cessation of exposure. Prior to lavage, the rats were exsanguinated after anesthesia with ketamine hydrochloride (Parke-Davis, NJ) and Innovar-Vet (Pitman-Moore, Washington Crossing, NJ), 50 mg/kg and 0.1 ml/kg, both given intramuscularly. Alveolar macrophages were

obtained by bronchoalveolar lavage (BAL), as described earlier [21]. Briefly, the lung was lavaged *in situ* repeatedly with 10 ml of warmed (37°C) calcium- and magnesium-free Hanks' balanced salt solution (HBSS; GIBCO, Grand Island, NY).

Culture of alveolar macrophages: The BAL cells of 3 to 5 animals were combined and washed twice with HBSS and once with RPMI-1640 medium (RPMI; GIBCO) in that order and resuspended in RPMI supplemented with 10% of heat-inactivated fetal bovine serum, 25 mM HEPES buffer and 60 µg/ml of gentamicin (RPMI-FBS). The number of cells and viability were assessed by trypan blue exclusion in a hemocytometer. BAL cells as AM were determined by morphological features on a Giemsa stained cytocentrifuge preparation then confirmed by peroxidase and non-specific esterase staining as previously described [20]. The BAL cells exhibited greater than 90% viability in all groups of rats. The proportion of AM obtained from BAL in all groups of rats ranged from 90 to 99%. The concentration of BAL cells was adjusted to 5, 10, 15, 20 × 10⁶ cells/ml. For culturing purposes, 0.1 ml of the suspension of BAL cells was added to 96 well microplates (Costar, Cambridge, MA) and incubated for 1 hr at 37°C in a humidified environment containing 5% CO₂. Non-adherent cells were then removed by simply washing the cultures 3 times with HBSS. After washing, 0.1 ml of RPMI-FBS was added to the adherent cell cultures. BAL adherent cells were cultured for 48 hr at 37°C in 5% CO₂. After 48 hr of culturing, the adherent cell populations always constituted at least 95% macrophages whose viability, based on the trypan blue exclusion test, always exceeded 95%. In one series of experiments, the culture supernatants were collected after 24, 48, 72, and 96 hr of culturing. The supernatants were frozen in aliquots at -70°C, until tested for chemotactic activity.

Fetal rat skin fibroblast and culture: Fetal rat skin fibroblast CRL1213 was obtained from American Type Culture Collection (Rockville, MD) and maintained in Dulbecco's minimal essential medium (DMEM; GIBCO) supplemented with 10% of heat-inactivated fetal bovine serum, 25 mM HEPES buffer and 60 µg/ml of gentamicin (DMEM-FBS) until the monolayers were almost confluent. Confluent monolayers of fibroblasts from 3 to 4 day fresh culture were rinsed twice with phosphate buffered saline, detached from the culture dishes with 0.25% trypsin and 0.02% EDTA solution (GIBCO) and resuspended in DMEM. The concentration of fibroblast was adjusted to 1 × 10⁶ cells/ml in DMEM. Trypan blue exclusion tests showed 95% viable cells.

Fibroblast chemotaxis assay: Culture supernatants of AM were assayed for chemotactic activity against fibroblast CRL1213 as responding cells, by using a microchemotaxis assembly with 48 wells (Neuroprobe Inc., Bethesda, MD), as described previously [21]. Briefly, culture supernatants were placed, in triplicate, in the bottom wells of the chemotaxis assembly. Gelatin coated 8 µm pore-size polycarbonate filters (Nucleopore Corp.,

Pleasanton, CA) [32], were then applied, whereupon 50 µl of responding cells were added to the top chambers. The assembly was incubated for 5 hr at 37°C in a humidified environment containing 5% CO₂. Thereafter, the filters were removed, fixed in Diff-Quik fixative (American Scientific Products, McGaw Park, IL), and subsequently stained with a Diff-Quik stain set. Chemotactic activity was evaluated by the number of fibroblasts migrating completely through the filter in 10 high power fields (2.462 mm²) at a magnification of X400 under the microscope (Wild 20, objective X40; eyepiece X10, Leica Inc., Deerfield, IL). Results were expressed as the mean ± S. E. M. for 3 or more separate experiments, after subtracting the number of cells that migrated toward the chemotaxis buffer alone. Purified human fibronectin (12.5 µg/ml in DMEM; Collaborative Research Inc., Lexington, MA) was used as "Positive control? chemoattractant for fibroblasts.

Evaluation of chemokinetic activity in AM supernatants: In order to assess whether culture supernatants produce directed migration of fibroblasts (chemotaxis) or increased random migration (chemokinesis), some samples were assayed in a "checkerboard" fashion as described previously [21].

Partial characterization of the chemoattractant: To determine whether the chemoattractant was dialyzable, the supernatants from Fischer-344 rats were dialyzed by using Spectrapor membrane (Spectram Industries, Los Angeles, CA), with a molecular weight cut-off of 3,500 for 18 hr at 4°C against DMEM-FBS. Thermostability was evaluated by subjecting the supernatants to heating at 56°C for 30 min and at 100°C for 10 min. Trypsin sensitivity of the supernatants was determined by adding 0.1 ml of a saline suspension of insoluble trypsin bound to cross-linked agarose (Sigma, St. Louis, MO) to 0.9 ml of the supernatants at a final concentration of 0.5 U/ml, and incubating for 1 hr at 37°C. To block trypsin activity, the supernatants were incubated with 10⁻³M phenyl-methylsulfonyl-fluoride (PMSF) for 1 hr at 37°C. After centrifuging at 1,500 rpm for 10 min, the supernatants were then collected. However, equivalent volumes of saline and PMSF were substituted for trypsin in control experiments. Chemotactic activities in these treated samples were compared to non-treated samples.

RESULTS

Optimal AM concentration on release of the migration factor for fibroblasts: When the concentration of AM collected from Fischer-344 rats added to the culture was varied from 1 × 10⁵ to 20 × 10⁵ cells/well, enhancement of migration activity for fibroblasts correlated with the number of AM was shown in the supernatant from both the asbestos-exposed groups and the sham-exposed group (Fig. 1). Therefore, the AM supernatants of all three groups yielded maximum migration activity for fibroblasts at the 20 × 10⁵ cells/well concentration, and the subsequent concentration of AM added to the culture was 20 × 10⁵

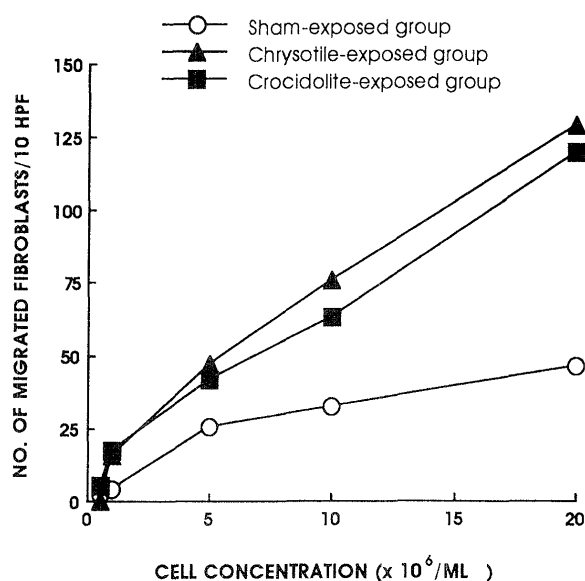


Fig. 1. The effect of alveolar macrophage concentrations on the migration activity towards fibroblasts in the culture supernatants from crocidolite-exposed, chrysotile-exposed and sham-exposed control rats in Fischer-344 strain. The supernatants were collected from 24 hr serum-supplemented cultures. Dulbecco's minimal essential medium served as a background. Mean \pm SEM of 4 experiments after subtracting the background (HPF=high power fields).

cells/well.

Optimal incubation time on release of the migration factor for fibroblasts: When AM from Fischer-344 rat were cultured for periods ranging from 24–96 hr, their supernatants yielded maximum migration of fibroblasts after 48 hr in culture from both asbestos-exposed groups and after 72 hr in the sham-exposed group (Fig. 2). In subsequent culture of AM, therefore, the supernatants from all groups were collected at 48 hr of culturing period.

Effects of asbestos inhalation on release of migration factor for fibroblasts in two different rat strains: Production of this migration factor(s) for fibroblasts has been observed in two different rat strains (Table 1). The migration activities for fibroblasts in Fischer-344 rats were significantly higher than in ACI rats ($p < 0.01$). However, in both rat strains, the migration activities for fibroblasts in the sham-exposed group were lower than in the asbestos-exposed groups. The migration activities for fibroblasts were significantly greater after crocidolite-exposure than after sham-exposure ($p < 0.01$) in both of the rat strains. A significant effect was only observed after chrysotile exposure ($p < 0.05$) in ACI rats. There were no significant differences between the migration activities for fibroblasts in the supernatants from both asbestos-exposed groups in both of the rat strains ($p > 0.05$).

Evaluation of chemokinetic activity in AM supernatants: As shown in Table 2, enhanced migration occurred in positive gradients of the supernatants from asbestos-exposed and sham-exposed rats. These indicated that the migration of fibroblasts was due to chemotaxis rather than

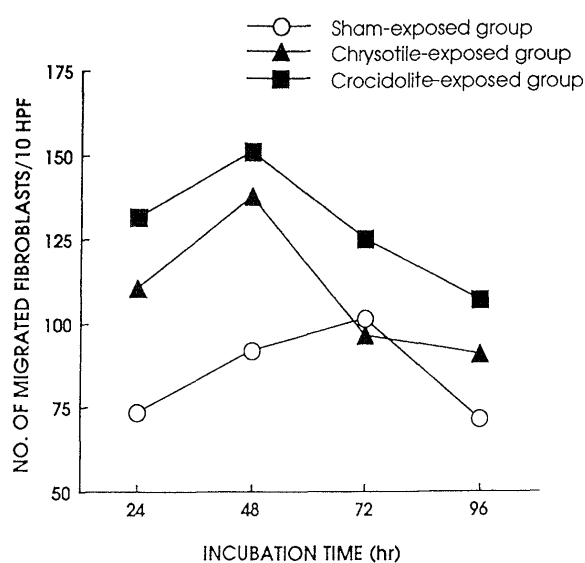


Fig. 2. The effect of incubation time in the culture on the migration activity towards fibroblasts in the culture supernatants from crocidolite-exposed, chrysotile-exposed and sham-exposed control rats in Fischer-344 strain. Supernatants were collected from cultures containing 2×10^6 added alveolar macrophages. Mean \pm SEM of 6 experiments after subtracting the background (HPF=high power fields).

Table 1. Alveolar macrophage derived migration activity towards fibroblasts in the culture supernatants from crocidolite-exposed, chrysotile-exposed and sham-exposed control rats in ACI and Fischer-344 strain

Experimental groups	Number of fibroblasts migrated	
	ACI	Fischer-344
Sham-exposed group	56.0 \pm 26.8	473.8 \pm 223.1†
Chrysotile-exposed group	124.5 \pm 74.7*	590.4 \pm 184.9†
Crocidolite-exposed group	124.8 \pm 58.5**	713.6 \pm 229.0†**
Human fibronectine	538.5 \pm 69.4	

Supernatants were collected from 48 hr cultures containing 2×10^6 added alveolar macrophages. Purified human fibronectin (12.5 μ g/ml in Dulbecco's minimal essential medium) served as a positive control. Values represent the numbers of fibroblasts migrated (mean \pm SEM)/10 high power fields in 7 experiments after subtracting the background. The chemotactic activities in Fischer-344 rats were significantly higher than in ACI rats ($p < 0.01$). †: ACI rat vs. Fischer-344 ($p < 0.01$). *: sham-exposed group vs. asbestos-exposed group ($p < 0.05$). **: sham-exposed group vs. asbestos-exposed group ($p < 0.01$).

to chemokinesis. However, in the crocidolite-exposed group, migration of fibroblasts exposed to uniform concentration of supernatants, as shown in diagonal bars, was increased according to the increase in the supernatants. Therefore, these AM supernatants contained chemokinetic activity for fibroblasts.

Partial characterization of the chemoattractant: The supernatants from 3 rat groups had their chemotactic activity significantly reduced ($p < 0.01$) by dialysis treat-

Table 2. Checkerboard assay on migration of culture supernatants from alveolar macrophages to fibroblasts

Dilution of supernatants		Dilution of supernatants above filter			
below filter		0	1/3	2/3	Undiluted
Sham-exposed group	0	159±35.4	108±39.7	86± 14.7	61± 22.3
	1/3	134±33.3	95±9.0	111± 9.5	81± 13.3
	2/3	262±58.0	101±11.7	142± 42.0	69± 8.5
	Undiluted	289±21.5	224±46.1	307± 84.5	242± 64.0
Chrysotile-exposed group	0	73±18.2	79±22.0	102± 52.5	46± 10.6
	1/3	83±25.8	105±17.3	101± 12.5	75± 26.2
	2/3	142± 5.0	187±48.3	182±108.0	185± 10.5
	Undiluted	380±58.3	375±15.3	360± 15.4	314±126.3
Crocitolite-exposed group	0	227±68.5	233±67.5	283± 91.9	263± 65.3
	1/3	322±137.5	318±53.3	289± 71.6	237± 27.2
	2/3	364±35.2	367±50.5	371± 85.3	466± 87.6
	Undiluted	478±73.3	454±17.9	536±134.0	812± 19.2

Values represent numbers of migrated fibroblasts (mean±SEM)/10 high power fields in triplicate experiments. Values below and to the left of the diagonal indicate chemotactic migration in a positive concentration gradient. Values within the diagonal indicate chemokinetic migration.

Table 3. The effect of dialysis treatment on the chemotactic activity in the culture supernatants from crocidolite-exposed, chrysotile-exposed and sham-exposed control rats of the Fischer-344 strain

Treatments	Number of fibroblasts migrated		
	Sham-exposed group	Chrysotile-exposed group	Crocitolite-exposed group
Undialysed	300.0±135.1	755.0±179.9	1013.0±101.5
Dialysed	109.3±94.5**	148.3±21.4**	644.0±62.2**

Supernatants from 48 hr cultures containing 2×10^6 added alveolar macrophages were dialyzed for 18 hr at 4°C against Dulbecco's minimal essential medium supplemented with 10% fetal bovine serum. Values represent the numbers of fibroblasts migrated (mean±SEM)/10 high power fields in 3 experiments after subtracting the background. **: Undialysed vs. dialysed ($p < 0.01$).

Table 4. The effect of heat treatment on the chemotactic activity in the culture supernatants from crocidolite-exposed, chrysotile-exposed and sham-exposed control rats of the Fischer-344 strain

	Number of fibroblasts migrated		
	Sham-exposed group	Chrysotile-exposed group	Crocitolite-exposed group
Unheated	300.0±135.1	755.0±179.9	1013.0±101.5
56°C (30 minutes)	49.7±24.1**	41.3±4.2**	87.3±11.6**
100°C (10 minutes)	3.0±5.3**	4.5±3.7**	3.7±3.2**

Supernatants from 48 hr cultures containing 2×10^6 added alveolar macrophages were heated at 56°C for 30 min or 100°C for 10 min. Values represent numbers of fibroblasts migrated (mean±SEM)/10 high power fields in 3 experiments after subtracting the background. **: Unheated vs. heat treatments ($p < 0.01$).

Table 5. The effect of trypsin treatment on the chemotactic activity in the culture supernatants from crocidolite-exposed, chrysotile-exposed and sham-exposed control rats of the Fischer-344 strain

Treatments	Number of fibroblasts migrated		
	Sham-exposed group	Chrysotile-exposed group	Crocitolite-exposed group
No trypsin	581.0±91.0	754.0±144.0	967.0±301.0
Trypsin (0.5 u/ml)	67.0±35.0**	58.0±28.0**	64.0±43.0**
Trypsin+PMSF	574.0±30.0	696.0±107.0	911.0±73.0
PMSF	620.0±65.0	754.0±74.0	981.0±88.0
Normal saline	672.0±87.0	788.0±23.0	989.0±116.0
Human fibronectin	581.0±58.0		
Medium alone	58.0±29.0		

Supernatants from 48 hr cultures containing 2×10^6 added alveolar macrophages were treated with 0.5 U/ml of insoluble trypsin, trypsin added 0.001 M phenyl-methyl-sulfonyl-fluoride (PMSF), PMSF alone or normal saline at 37°C for 1 hr. Values represent the numbers of fibroblasts migrated (mean±SEM)/10 high power fields in 3 experiments. **: No trypsin vs. trypsin treatment ($p < 0.01$).

ment (Table 3), indicating that the factor was partially dialyzable.

Following heat treatment at 56°C for 30 min, the chemotactic activities in sham-exposed, chrysotile-exposed and crocidolite-exposed groups were strikingly reduced to approximately 16.6 and 9%, respectively ($p < 0.01$). Their chemotactic activities also completely disappeared when the supernatants were heated to 100°C for 10 min (Table 4). Therefore, heat treatments of the chemoattractants indicated that these are a thermolabile substance.

The trypsin treatment of the supernatants from all of

the rat groups for 1 hr at 37°C reduced their activities to as low as the background level (Table 5). These effects of the trypsin treatment were blocked by adding PMSF to the trypsin solution. Also the PMSF and the saline treatment did not affect the chemotactic activities, so the chemoattractant was thus shown to be a trypsin-sensitive protein.

DISCUSSION

In this study we demonstrated that asbestos inhalation in the 2 different rat strains was associated with enhanced release of a chemoattractant for fetal rat skin fibroblasts from AM. Maximal chemotactic activities of asbestos-exposed and sham-exposed rats were detected in the supernatant of the culture medium after 24 and 48 hr culture. In both rat strains, however, the chemotactic activities in asbestos-exposed animals were greater than in sham-exposed animals. The chemoattractant effects were significantly greater after crocidolite-exposure than after sham-exposure in both of the rat strains. Moreover, significant effects were only observed after chrysotile exposure in ACI rats. Even though there were differences between the fiber lengths, physicochemical properties, and biological potentials of the two types of asbestos [2, 7, 29], it was an unexpected finding that there were no significant differences between the chemotactic activities of the supernatants from both asbestos-exposed groups in both of the rat strains, as comparable amounts of chemotactic activities for AM were detected in the supernatant from the asbestos-exposed rats [21]. Although greater activities were noted in the supernatants from the crocidolite-exposed rodents than in those from the chrysotile-exposed ones, the differences were not statistically significant.

The culture supernatants from Fischer-344 rats exhibited significantly higher chemotactic activities than those from ACI rats. Other investigators, using either undefined or dissimilar strains of rats, have indicated that any extensive asbestos-related fibrosis can be induced effortlessly in the rats [9, 15, 37]. It is possible that different degrees of fibrosis may correlate to the extent of chemotactic activities towards fibroblasts. Therefore, this difference may possibly relate to genetic differences in the susceptibilities of different rodent strains to pulmonary fibrosis within different strains, as strain variation has been shown to determine susceptibility to bleomycin-induced pulmonary fibrosis in mice [34].

There were a greater number of fibroblasts migrated in the supernatants from Fischer-344 rats than in those from ACI rats. The fibroblasts incubated in the presence of increasing chemotactic factor gradients from Fischer-344 rats exhibited increased migration; there were dose-dependent effects and chemokinetic effects at higher concentration gradients. Therefore, the assay conditions for chemotaxis were accordingly designed to compare the activities in the supernatants of AM from both strains of rats. These conditions, however, were suitable for the supernatants of AM from the ACI rats, but not for the

supernatants of AM from the Fischer-344 rats. The fibroblasts were overly incubated and/or stimulated in the supernatants of AM from the Fischer-344 rats. Thus, true chemotactic activities may have been masked by chemokinetic activities. Many materials that stimulate chemotaxis also stimulate chemokinesis and these stimulatory effects were dependent on the concentration of chemotactic factors placed in the chemotaxis chambers [38]. In our studies, chemoattractants were released from cultured AM, but not only from serum supplemented culture medium. This seems probable, because AM constituted at least 95% of the BAL adherent cell populations derived from all three experimental groups and chemoattractants increased with culture time and the number of BAL cells added in the culture. Furthermore, Kagan *et al.* [21] reported that true chemotactic activities for AM were detected in similar supernatants of AM culture. Thus, our stimulatory effects are associated with chemotactic activities, rather than with chemokinesis. To confirm these chemotactic activities, in further studies it will be necessary to carry out chemotaxis analyses under suitable conditions, i.e. serum free conditioned medium for AM culturing, different responding cells, and shorter incubation time.

A number of AM-derived chemotactic factors have been described in several animal species including primates [16, 25, 33], rabbits [10, 14], guinea pigs [8, 17] and rats [21]. Such chemotactic activities have been known for neutrophils [8, 10, 14, 16, 17, 25, 33], monocytes [25], AM [21] and fibroblasts [33]. It is believed that the release of these AM-derived chemoattractants may be important in the pathogenesis of various interstitial lung disorders. Kagan *et al.* [21] have demonstrated, again, the enhanced release of the chemotactic factor for the rat AM after asbestos inhalation. Enhanced release of this chemoattractant after asbestos inhalation may explain the AM accumulation at the sites of asbestos deposition in the lungs in early lesions [5, 6, 22, 36]. These newly recruited AM may also induce fibroblast activation and fibrogenesis via AM secretory products [1, 4, 12, 13, 24]. Moreover, our data indicated that both amphibole and serpentine asbestos inhalation appear to be associated with enhanced release of an AM-derived chemoattractant for fibroblasts. It is possible, however, that the fibroblasts may have recruited themselves to the sites of the asbestos-mediated pulmonary injury. In this regard, with this in mind, it is pertinent that stimulated AM release the glycoprotein, fibronectin, which is a potent fibroblast chemoattractant [28, 31, 33, 35]. There are numerous macrophage-derived cytokines, such as interleukin-1 [13,24], transforming growth factor- β [23,26], alveolar macrophage derived growth factor [4] and platelet-derived growth factor (PDGF) [1,27] which could influence the chemotaxis of fibroblasts. In this connection Osornio-Vargas *et al.* [30] suggested that rat lung fibroblasts were chemotactic to the rat alveolar macrophage-derived PDGF which was produced after stimulation with carbonyl iron particles and asbestos fibers and reported that chemotactic activities

were observed in two fractions with molecular masses of 150,000 and over 200,000 daltons which complexed to an α -macroglobulin. We have come to understand, therefore, that the chemoattractant is thermolabile, trypsin-sensitive and partially dialyzable substance. Similar observations, however, were previously recorded by Kagan *et al.* [21]. They also reported that the molecular weights of the chemoattractant for the AM corresponded to 13,000–15,000 daltons, 23,000–26,000 daltons and 45,000–46,000 daltons in SDS-polyacrylamide gel electrophoresis. The molecular weight was not determined in the present work. Further studies will be necessary to determine the molecular weights of the macrophage-derived protein.

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