Alveolar macrophage kinetics and multinucleated giant cell formation after lung injury

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Abstract: Multinucleated giant cells (MGC) are a prominent feature of some chronic inflammatory states in the lung. These cells are formed by macrophage fusion, but how this process relates to the kinetics of alveolar macrophage (AM) production and proliferation is not clear. In this serial study, we compare AM kinetics and MGC formation after instilling carbon, silica, asbestos, bleomycin, and saline into the lungs of mice. Animals were killed up to 16 weeks later with [^{3}H]thymidine injected 1 h before death. Counts of AM and MGC were carried out after bronchoalveolar lavage, and cell labeling was assessed by autoradiography. All test substances induced an inflammatory response with equal AM numbers recovered up to 2 weeks. Subsequently, the number returned to normal after carbon but remained elevated in the other groups. After carbon the lung structure was normal, there was no increase in AM label, and no MGC formed. Bleomycin-injected lungs progressed to fibrosis with only a brief, small increase in AM labeling and no MGC formation. After silica, and particularly asbestos, the lungs showed fibrosis, and many granulomas with large MGC were seen. Lavaged AM from these lungs showed a significant increase in DNA synthesis after 2 weeks, followed by higher numbers of MGC, none of which were labeled. Labeled AM tended to be free of particles, whereas MGC after 4 weeks contained many particles. The results indicate a relationship between AM proliferation and fusion, whereby AM growth appears to be prerequisite for cell fusion and MGC formation as a feature of granulomatous disease. *J. Leukoc. Biol.* 59: 534–538; 1996.

Key Words: *granuloma · asbestos · silica*

INTRODUCTION

The alveolar macrophage (AM) is believed to play a central role in the evolution of pulmonary fibrosis after different types of lung injury. Various growth factors for fibroblasts have been identified as products of AM in response to particle inhalation or instillation [1–4] and to the administration of drugs such as bleomycin [5]. An early lung response to this drug or to particles, such as silica and asbestos, or even to an inert particle such as carbon, is an inflammatory cell efflux including an increase in AM numbers. While the lung structure remains normal after carbon, the response to silica, asbestos, or bleomycin leads to a fibrotic lung from which lavaged AM and isolated interstitial macrophages have been shown to produce factors, such as platelet-derived growth factor, that stimulate fibroblast growth [1, 3, 6]. There is, however, a difference in pulmonary pathology in these lung reactions. While bleomycin administration induces diffuse interstitial fibrosis [7], silica and asbestos also induce granuloma formation with many macrophages and multinucleated giant cells (MGC) [4, 8, 9]. Although it is recognized that these cells form by macrophage fusion [10], it is not clear if this happens with mature cells or if the process follows cell division and/or activation, although there is evidence that, in vitro, macrophage fusion may follow cell proliferation [11]. To determine whether this relationship occurs in vivo, we have compared macrophage kinetics and the incidence of giant cell formation in examples of lung inflammation that resolve completely (after carbon), that lead to interstitial fibrosis (after bleomycin), and that lead to fibrosis with MGC and granuloma formation (after silica and asbestos).

MATERIALS AND METHODS

All experiments were carried out in 25-g male Swiss Webster mice. Full details of each procedure have been published by us previously [8, 9, 12, 13], but without the macrophage and giant cell kinetics, which we describe here. (1) Intratracheal (IT) silica: under mild Nembutal anesthesia, mice were injected with 1 mg quartz suspended in 0.1 ml saline [8]. (2) IT crocidolite: mice were injected with 0.1 mg of the UICC mixed fiber sample [9]. (3) Carbon: mice were injected IT with 1 mg of a carbon black suspension [12]. (4) IT bleomycin: mice received 0.15 mg bleomycin in 0.1 ml saline [13]. (5) IT saline: this group of mice received 0.1 ml saline and was used as a control group for all of the above treatments.

In each experimental group, mice were killed by barbiturate overdose in groups of four at intervals up to 16 weeks; all animals received 2 μCi/g tritiated thymidine (^{3}HT) 1 h before death. A tracheotomy was

Abbreviations: MGC, multinucleated giant cells; AM, alveolar macrophages; IT, intratracheal; PMN, polymorphonuclear leukocytes; CSF, colony-stimulating factor.

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performed, and the lungs were washed four times with 1.0 ml saline. The lavage fluid was pooled for each animal and the total number of cells was determined after counting by hemocytometer. Several cytospin preparations were made; two were stained and differential cell counts were made on 500 cells per animal. The numbers of AM, polymorphonuclear leukocytes (PMN), and other cell types were calculated. This included multinucleated giant cells (MGC), defined as any cell with more than two nuclei. Two more cytospins per animal were used for autoradiography using Kodak NTB2 emulsion. After exposure for 2 weeks and developing, the percentage of labeled AM for each animal was determined after counting 300 cells per slide. For cell counts and percent labeled AM, mean values ± SE were calculated for each group and compared with saline-injected controls at the same time; significance was determined with the Student's t-test.

After lavage, the lungs were inflated with buffered gluteraldehyde and processed for embedding in glycol methacrylate. Lung morphology was assessed in 1-μm sections. A sample of each lung was also prepared for electron microscopy.

RESULTS

Cell counts

The total cells recovered by lavage were slightly increased 0.5 wk after IT saline but otherwise were at the time 0 or non-injected control levels, which are the only values shown to simplify presentation. Each injected substance induced a comparable inflammatory response in the first 2 weeks as macrophage numbers increased three to four times (**Figs. 1** and **2**). A prominent PMN response was also found as described previously [8, 9, 12, 14]. After carbon injection, the cell numbers in lavage fluid returned to normal after 2 weeks (Fig. 1). In lung sections, AM containing carbon were still evident for several weeks longer but there was little PMN response and the lung structure was normal. After instilling bleomycin, the AM numbers remained above normal to 16 weeks (Fig. 1) and the lung showed areas of diffuse interstitial fibrosis as previously described [7, 13].

The macrophagic response to silica and asbestos was similar. After an early peak in AM recovered by lavage, the numbers fell somewhat but remained about twice control values to 16 weeks (Fig. 2). The lungs after silica showed areas of fibrosis and several granulomas were observed, particularly in peribronchiolar locations. Some MGC with

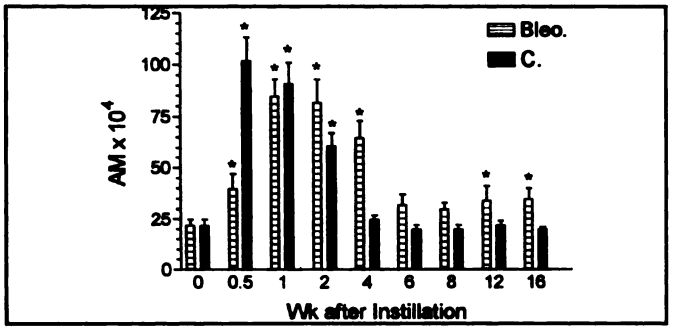

Fig. 1. Number of alveolar macrophages (AM) recovered by lavage up to 16 weeks after instilling carbon (C) or bleomycin (Bleo) to the lung. *P< 0.05 compared to saline control.

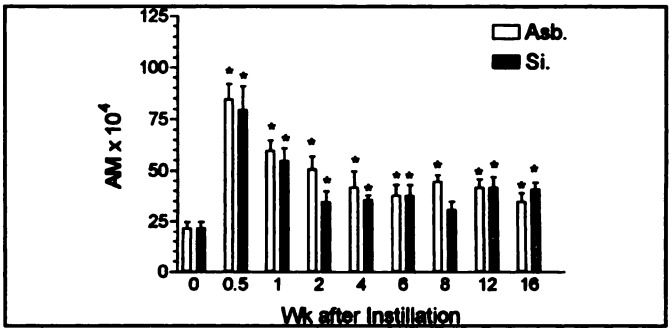

Fig. 2. Number of alveolar macrophages (AM) recovered by lavage up to 16 weeks after instilling silica (Si) or crocidolite asbestos (Asb) to the lung. *P< 0.05 compared to control.

silica were identified in these areas, which became more fibrotic with time. Granulomas were more prominent after asbestos, and the lungs contained many large MGC in alveoli and in the interstitium (**Fig. 3**).

The number of MGC in the bronchoalveolar lavage fluid was determined for each experimental group. MGC were never found in control animals or in animals that received carbon or bleomycin. They were found, however, by lavage after silica and particularly after asbestos from 2 weeks onward (**Fig. 4**). The number recovered by lavage peaked

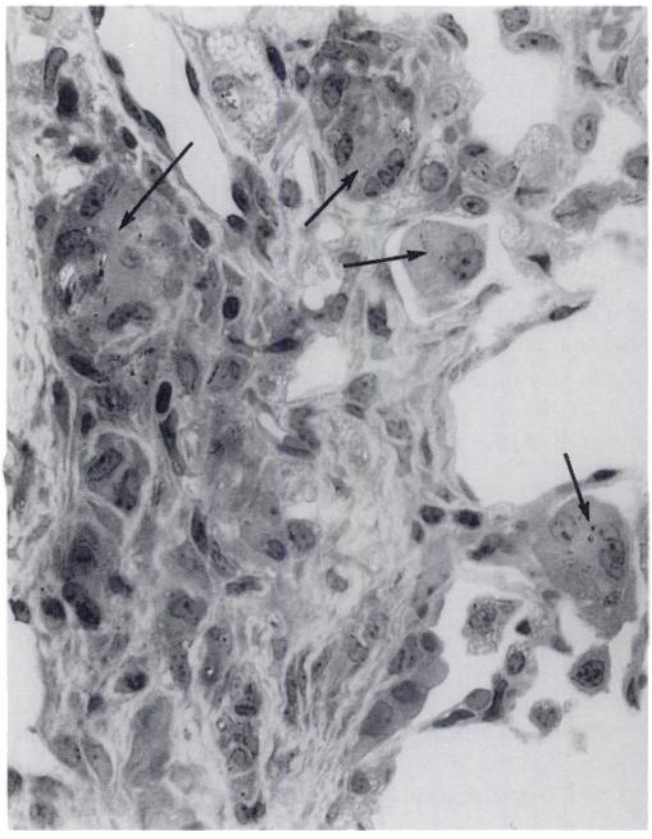

Fig. 3. Lung section 12 weeks after instilling asbestos shows an interstitial granuloma with multinucleated giant cells (arrows) containing many asbestos fibers. Similar cells are also seen in the alveoli (arrows). Magnification is ×850.

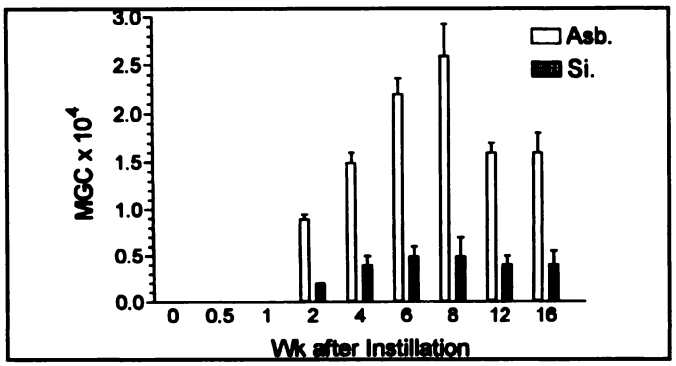

Fig. 4. Number of multinucleated giant cells (MGC) recovered by lung lavage up to 16 weeks after instilling silica (Si) or asbestos (Asb). All values shown are significant because controls were zero.

at 8 weeks, although at later times the number counted may underestimate the actual total, since some very large MGC were seen in sections and many seem too large to be cleared normally or to be lavaged from alveoli (Fig. 3). These cells were confirmed as MGC rather than aggregates of AM when examined by electron microscopy. In the earlier stages, the giant cells had few phagocytized particles (**Fig. 5**), whereas most contained many particles or fibers at later times (Fig. 3).

Autoradiography

The percentage of thymidine-labeled AM in control mice was always ~0.5 and this level was unchanged in mice that received IT carbon. The labeling of AM doubled at 1–4 weeks after bleomycin then fell to control values (**Fig. 6**). A slightly higher proliferative response of AM was found in cells lavaged from lungs after IT silica, and labeling returned to normal by 6 weeks (**Fig. 7**). The highest labeling percentage was found after asbestos, whereby ~3% of AM were labeled in the period 2–6 weeks. At this time, labeled cells tended to have few if any phagocytized fibers compared with other lavaged AM (**Fig. 8**), and although the percentage of labeled cells fell after 6 weeks, it was still above normal to the end of the study.

Giant cells were also examined for thymidine incorporation but labeling of MGC was never seen in any of the cytospins.

DISCUSSION

Chemotactic and mitogenic factors present in the alveoli are responsible for an increase in the number of macrophages following the deposition of particles into the lung or the initiation of cell injury. Macrophage numbers increase through migration of blood monocytes plus a component of local cell proliferation [12]. In the present study, the number of AM recovered by lavage in the first 2 weeks after such diverse substances as carbon, bleomycin, silica, or asbestos was similar and so is not predictive of any subsequent pulmonary pathology. However, after 4 weeks, a continuing inflammatory response with increased AM correlates with the development of pulmonary fibrosis, and AM lavaged from lungs after silica, bleomycin, and asbestos have been shown to produce various cytokines known to stimulate fibroblast proliferation [3, 5, 6].

The lung pathology is different when comparing the long-term response to silica or asbestos with that seen after bleomycin. Bleomycin induces interstitial fibrosis in which alveolar and interstitial macrophages may appear foamy but are exclusively mononuclear [7, 14]. In contrast, after silica and especially crocidolite asbestos, fibrosis is complemented by a granulomatous response and many MGC are seen in alveoli and in the interstitium. These have been described previously as foreign body giant cells and have been attributed to fusion of macrophages [10]. However, the kinetics of this process, and how it relates to cell proliferation and activation in vivo have not been fully addressed. Our results show that MGC do not form purely as a consequence of the phagocytic process, since no MGC were found after administering carbon. Also MGC did not form during acute inflammation as none were seen up to 2 weeks after administering any of the agents used in this study. Giant cells were seen in large numbers only in the chronic stages, from 4 weeks onward, after silica and asbestos.

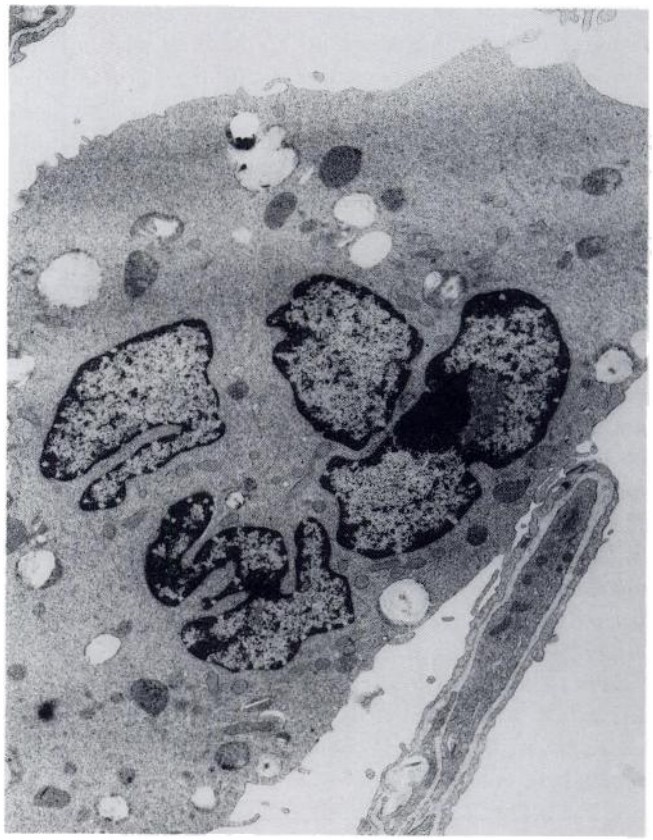

Fig. 5. Electron micrograph of an alveolar area containing a multinucleated giant cell 4 weeks after instilling asbestos. The cell contains little or no phagocytized material at this stage. Magnification is ×5500.

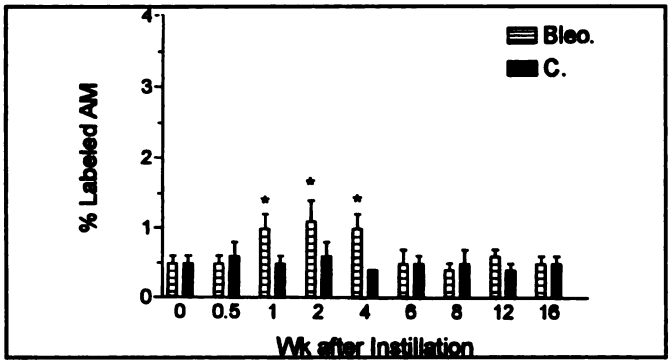

Fig. 6. Percentage of [^{3}H]thymidine-labeled alveolar macrophages recovered by lavage after instilling carbon (C) or bleomycin (Bleo) to the lung. *$P < 0.05$>control.

The formation of MGC appears to be related to proliferation of AM, in that the peak in MGC number occurs subsequent to the phase of maximal AM division. Labeled AM were cells with few phagocytized particles, suggesting an immature cell responding to a mitogenic stimulus. It is generally believed that the mature, functioning AM is not likely to divide and a very low mitotic rate in situ has been observed here and in other studies [11, 12]. In addition, none of the nuclei of MGC was ever labeled, indicating that these cells have no mitotic activity. They do, however, show continuing functional properties in that increased phagocytosis with time was demonstrated, and continued expression of AM surface markers has been shown on MGC [15]. The results are consistent with the idea that an inflammatory stimulus causes the migration of immature cells to the alveoli where some respond to a mitogen. Although the precise reason for the subsequent fusion of some of these cells is unknown, the resultant giant cells appear to differentiate and remain as functional derivatives of the mononuclear phagocyte series of cells.

Differences in monokine production between AM associated with fibrosis and cells from granulomatous lung have been shown before [4]. The proliferation of free AM can be induced by various colony-stimulating factors (CSF), and in culture, MGC are also formed [16], for example after the addition of interleukin-4 [17]. Although such factors have usually been thought to be produced by lymphocytes, it has been shown that lung fibroblasts also produce macrophage-CSF [18], so it is possible that these fibroblasts act in a reciprocal fashion by producing a growth factor for AM, while themselves responding to an AM-derived cytokine [3, 6]. Once AM growth is initiated, results of in vitro studies suggest an inverse relationship between fusion and proliferation [11]. It has been demonstrated that CSF stimulates macrophages to grow while also activating them. At this point, the addition of an agent such as 1α, 25-dihydroxyvitamin D_3 can result in the inhibition of cell division and the promotion of cell fusion [11].

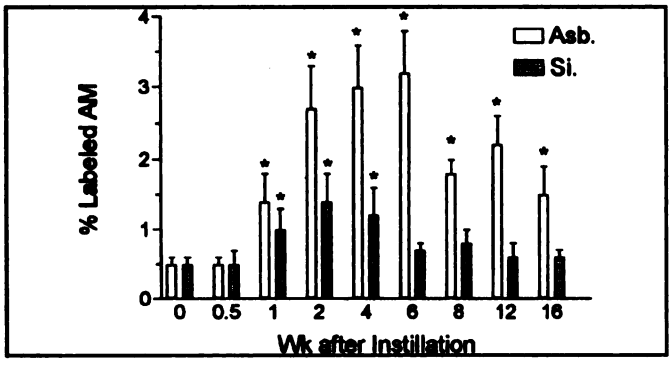

Fig. 7. Percentage of [^{3}H]thymidine-labeled alveolar macrophages (AM) recovered by lavage after instilling asbestos (Asb) or silica (Si) to the lung. *$P < 0.05$>control.

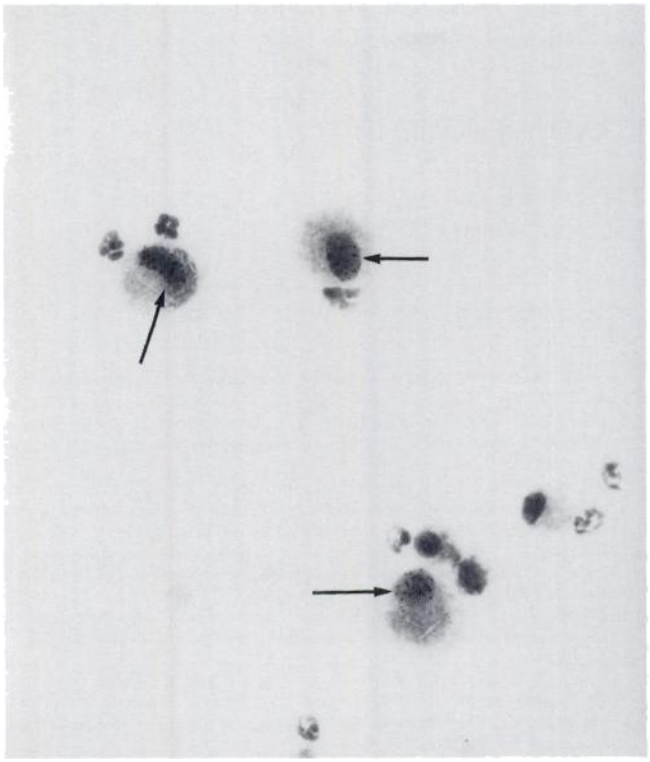

Fig. 8. Autoradiograph of cells lavaged from the lungs 2 weeks after instilling asbestos. Thymidine incorporation is demonstrated in some alveolar macrophages (arrows). Magnification is ×850.

We have now found evidence from in vivo studies that is consistent with the hypothesis that alveolar macrophage fusion occurs as a post-mitotic event. Although administration of bleomycin, silica, or asbestos to the lung results in activation and cytokine secretion by lavaged AM [3–5], a proliferative response in the AM population is only seen after silica and asbestos, implying that a factor such as CSF is produced in response to these particulates. The second stage of the process that leads to fusion of the proliferated cells may involve tumor necrosis factor α (TNF-α). This is produced in the lung in the chronic phase in response to asbestos [19], and a recent report implicates TNF-α involvement in the multinucleation of macrophages [20]. Because the present study indicates that cell fusion and MGC formation follow a phase of AM proliferation, the assessment of AM growth in a lavaged cell population may

be a useful marker for the subsequent development of granulomatous disease in the lung.

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