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REVIEW PAPERS

MACROPHAGE ACTIVITY IN ASBESTOS RELATED DISEASES

MACIEJ TARKOWSKI and PAWEŁ GÓRSKI

Clinic of Occupational Diseases, Institute of Occupational Medicine, Lodz, Poland

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Abstract. This paper indicates some immunological aspects of asbestos-related diseases and especially concerns the activity of macrophages – cells of immunological surveillance. Macrophages establish a very important population of cells which initiate or suppress specific immune response; they are responsible for effective T-cell activation, express antitumour activity. The process of lung fibrosis generated by the inhalation and deposition of asbestos fibres is also closely related to macrophage activity. An open question which is still to be resolved concerns asbestos-induced fibrosis; it may arise as a consequence of tissue injury and repair or change collagen synthesis. Another question is, to what extent macrophages may be protective cells and when they become undesirable? Since their overstimulation or damage in the case of chronic exposure to asbestos dust may be the reason of the increased release of inflammatory mediators, reactive oxygen intermediates which may in turn cause tissue injury, fibrosis or, in final effect, cancer. If so, we could then say that lung response (expressed by e.g. alveolar macrophage activity) to chemical insult may cause further damage to this tissue.

INTRODUCTION

It is known that asbestos fibres are recognized as hazardous pollutants in the occupational and general community environment. Inhaled asbestos fibres cause:

a) Asbestosis – interstitial fibrosis of the lung;
b) Cancer of the lung;
c) Mesothelioma of the pleura and peritoneum.

Address reprint requests to M. Tarkowski, Clinic of Occupational Diseases, Institute of Occupational Medicine, P.O. Box 199, 90-950 Lodz, Poland.

The harmful action of asbestos fibres involves pathological reactions whose final effect is well known only on pathomorphological bases. However, the process which lead to these morphological changes are still unclear, they involve many molecular and cellular pathological reactions, including, among others, immunological response. The important role which macrophages fulfil in the immune system and in asbestos-related diseases is unqestionable. Our knowledge, however, is still insufficient to correlate the immunological activity of macrophages with the harmful effect of asbestos dust.

Macrophages, as cells which first encounter inhaled mineral fibers, beside their primitive phagocytositic function, are important in:

1. antigen (Ag) presentation and T-cell activation;
2. differentiation and selection of certain cell populations;
3. suppressive reactions; and
4. anti-tumour defense mechanisms.

Since alveolar macrophages (AM) fulfil such a responsible role in the defense mechanisms of the host against invading factors and establish the first line of immune cells against asbestos dust, their activity must be the subject of intensive studies. Their impaired activity may be reflected in distemper regulation of immune response and finally diminished or abandoned tumouricidal activity.

Macrophages and T-cell activation

Macrophages, among the other so-called accessory cells, establish an important population of cells which initiate specific immune responses or indirectly suppress it. The process of immune response to specific Ag requires the presence of cells that exhibit class II of the major histocompatibility complex (MHC) molecules, produce an accessory signal (interleukin-1 (IL-1), process antigen and present it to specific T-lymphocytes, thus activating them. All these requirements are fulfilled by macrophages. The process of Ag presentation seems to be regulated through complex mechanisms and the final effect (immune response or unresponsiveness) depends on:

a) The presence of macrophages expressing II MHC molecules.

They may coexist in the same tissue with macrophages which do not express II MHC, therefore, they are not able to activate T-lymphocytes (21).

b) The competing Ag for MHC II molecules expressed by macrophages.

The process of Ag presentation involves its specific recognition through T-cell receptors which tolerate 10–20% of single aminoacid substitutions in complex Ag and MHC molecules expressed by accessory cells.

However, the MHC molecules in Ag presenting cells are tolerant in 80–90% of single aminoacid substitutions what makes possible that competing Ag would bind to these molecules, inhibiting the induction of specific T-cells (2).

c)The existence of co-stimulators and specific T-cell subpopulations.

Some T-cells may be activated if an appropriate co-signal is used (not IL-1 only) (30).

d) The presence of suppressor of T-lymphocytes.

e) The place where presentation takes place, since it is likely that there exist some "privileged" sites as the central nervous system or the eye where specialized populations of macrophages may temper or prevent immune responses (21).

f) Cytokines which enhance or diminish the process of T-cell activation.

However, the process of T-cell activation mediated by macrophages in humans exposed to asbestos fibres is poorly known, it could be predicted that this complex mechanism may be easily destroyed by asbestos fibres, causing pathologic reactions. It may be reflected in e.g. diminished production of the macrophage inhibition factor released by activated T-lymphocytes (13).

There is no strict confidence as to which type of fibre is the most hazardous, it is, however, accepted that their length is of great significance. From the studies of Allison et al. (3) it is known that macrophages are ingested by phagocytosis fibres not exceeding 5 μm, and they refer as to the short fibres, whereas long fibres, over 25 μm, are ingested only partially and the portion of them remains outside the cell. These long fibres are mainly suspected of many undesired effects as: leak of two lysosomal enzymes (beta-glucuronidase, beta-galactosidase) and cytoplasmic enzyme; lactic acid dehydrogenase (5,7). The increased secretion of these enzymes may be a sign of sub-lethal cell damage which was confirmed by Beck at al. (4) who showed that phagocytosis of long fibres was accompanied with increased metabolic energy and localized damage to the cell. The harmful activity of short fibres, however, is still not ruled out. From the studies of Yeager et al. (32) we know that naturally occurring short fibres (RG144) are more cytotoxic to macrophages than longer ones. The process of Ag presentation mediated by macrophages may be disregulated or completely abolished regardless of fibre type and length. Ag to be present to specific T-cell must be digested and then fixed to the cell surface. Since asbestos fibres are in most cases cytotoxic to macrophages they will not allow presentation to take place. The process of macrophage destruction may in effect cause injury of contiguous tissue through inflammatory reactions which may be the cause of the first stage in cancerogenesis; initiation.

Tumouricidal activity

Tumouricidal activity of macrophages is well-documented and can be mediated by a direct cytotoxic effect or in an indirect way in antibody-dependent cell-mediated cytotoxicity (ADCC) mechanism. In both cases, target cells are selectively bound to the macrophage cell surface and target cytolysis follows secretion of lytic effector molecules by the macrophage. Before cytolysis, macrophages must be activated by two signals acting in defined sequence. The same goes for effective killing and digesting of facultative intracellular parasites. The first signal, interferongamma, released from sensitive T-lymphocytes, primes responsive macrophages for subsequent response to a second signal which finally activates them to produce tumouricidal activity. The most commonly employed as a second signal are bacterial

lipopolysaccharides (LPS), listeria monocytogenes and muramyl dipeptide. Those signals activating macrophage-mediated tumour cytotoxicity are also effective in mediating the ADCC mechanisms. The importance of ADCC mechanisms could play some role in asbestos-exposed subjects, especially those who suffer from asbestos-related cancer. There is some evidence from the studies of Scheule et al. (23) that macrophage activity to release radical oxygen intermediates (ROI) is IgG-mediated and specific for asbestos fibres. This could indicate the role of antibodies in defense mechanisms against asbestos-induced cancer killing, mediated through the ADCC mechanisms. It is likely to appear, since xenobiotics, in contrast to viruses, can cause expression of different tumour determinants to which specific antibodies are needed. AM of healthy subjects exhibit a low level of antitumour activity unless treated by muramyl dipeptide or LPS. Those cells co-cultivated with three different tumour cell lines and activated with LPS release as many cytotoxic factors as AM incubated without tumour cells. Failure of AM to produce cytotoxic factors in response to a potent activator could restrict their role in defense of the lung (25). Perhaps asbestos fibres may interfere with an activating factor not allowing the full activation process.

As was mentioned above, in both mechanisms of tumoricidal activity, the effector mechanism is mediated through the release of cytotoxic factors. It was suggested that ROI, neutral protease, arginase, tumour necrosis factor (TNF) contribute to tumouricidal activity but the exact mechanism of effector cytotoxicity is still unclear. Recently, Miyakawa et al., (17) has shown the main role of TNF-like molecules in tumour-killing in contrast to ROI-dependant way. Other authors attribute the main role of tumour killing to neutral protease (1); others to the process of phagocytosis (19). Different effector mechanisms against tumour cells may arise from the heterogeneity of individual patients and heterogeneity of malignant tissue. Therefore it would be naive to suspect the immunological mechanisms against tumour cells to be identical or even similar in all patients with cancer.

Macrophages and fibrosis

It seems likely that production of one of the antitumour factors that is ROI under influence of asbestos fibres may cause undesirable effects which result in fibrosis of the lung. The process of increased ROI production, and in consequence, tissue injury expressed by membrane peroxidation, and damage to macromolecules, is believed to be caused mainly by long fibres which do not undergo complete phagocytosis (18).

Increased production of ROI by AM was reported in various animal models. Case et al. (6) showed increased superoxide anion production *in vitro* from bronchoalveolar lavage cells derived from the Syrian golden hamster. The same effect was found in mice by Goodglick at al. (8). The enhanced production of superoxide anion by AM caused by IgG opsonized asbestos fibres, was also detected in guinea pigs (23). The oxidative injury of the lung could be elevated additionally by polymorphonuclear leukocytes (PMNL), since macrophages

can produce a factor which induces their infiltration (16) or are defective in releasing a factor that inhibits migration and chemotaxis of PMNL (24). This is in agreement with Petruska et al. (20) who found altered bronchoalveolar lavage cell population characteristics of an acute inflammatory response after short-term exposure of rats to asbestos dust. Macrophages and chemo--attracted PMNL in sites of inflammation, being exposed to potent activators of oxygen radical production, may cause the injury of the lung tissue, and finally may initiate the process of fibrosis.

It is well established that inflammatory process is elevated by cytokines among which are those released from monocyte/macrophage cell lineage. Those cytokines e.g. TNF-alpha are well known inducers of ROI production (14). The process of extensive ROI production, which leads to injury, and may in turn cause increased IL-1 production, was shown by Gougerot--Pocidalo et al. (9). This process was ascribed to human monocytes and we can only predict that the same effect goes for the cells of the same lineage i.e. macrophages in the site of the lung injury indirectly caused by asbestos fibres. Enhanced IL-1 activity may in turn increase inflammatory response and cause other changes in regulation mechanisms of immunological response. This findings are in agreement with previous studies (10) which indicated the enhanced phytohaemaglutynine-stimulated increase of IL-2 production by peripheral blood mononuclear cells in sites of tissue injury. The explanation, mentioned by authors, is now evident and indicates that the increased IL-2 production was the result of enhanced IL-1 activity. However, the proliferation of lymphocytes was decreased, since oxygen radicals interfere in early events of cell cycle. This may explain the diminished number of T-cells in patients exposed to asbestos dust observed by Kagan et al. (13).

The process of lung tissue injury and subsequent fibrosis seems to be mainly caused by increased level of ROI generated by cells during incomplete phagocytosis of long fibres. An elevated production of ROI may also arise from decreased activity of antioxidant enzymes which protect against toxic oxygen radicals during normal metabolism and after oxidative insults (27), as is shown in Fig. 1.

There is no evidence that asbestos fibres cause destruction of protective systems of enzymes; however from the studies of Mossman and Landesman (18) we know that these toxic agents may cause peroxidation of membranes and damage to the macromolecules of airway epithelial cells. This may arise from excessive production of ROI and, on the other hand, diminished or abandoned activity of the protective enzyme system.

The process of lung fibrosis shown here as a consequence of tissue injury and repair may also be a cause of direct effect on collagen synthesis. There is also the possibility that both the mechanisms concerned (injury and repair and increased collagen synthesis), are active in the process of lung fibrosis through cellular interactions.

Cellular complexity of the lung raises a question of cellular interactions in the induction of fibrosis. The hypothetical mechanism of cellular interactions leading to lung fibrosis is shown in Fig. 2. The most likely process of

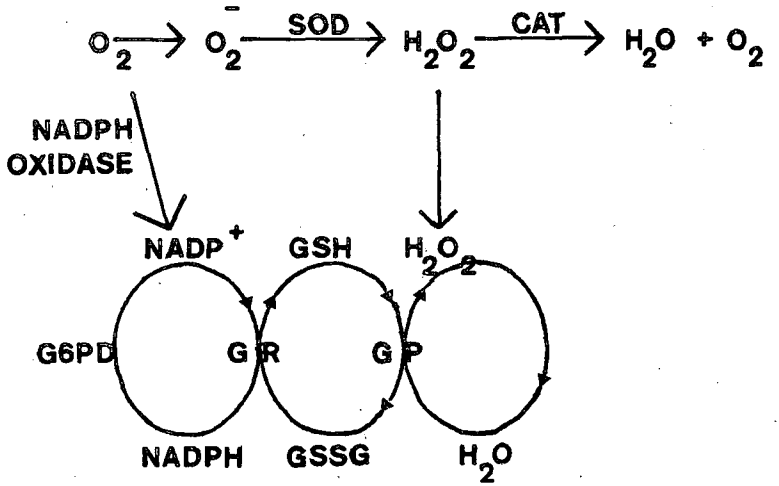

Fig. 1. The pathways of detoxication of ROI mediated by antioxidant enzymes:
a) SOD – Superoxide dismutase which dysmutates two molecules of superoxide anion to form hydrogen peroxide and oxygen molecule,
b) CAT – Catalase. It catalyzes the reaction which forms, from two molecules of hydrogen peroxide, two molecules of water and one molecule of oxygen,
c) G6PD – Glucose-6-phosphate dehydrogenase which catalyzes the reaction of oxidation of glucose-6-phosphate to 6-phosphoglucolactone with concomitant reduction of nicotinamide adenine dinucleotide phosphate (NADP) to its reduced form NADPH,
d) GP – Glutathione peroxidase. It catalyzes reaction of oxidation of Glutathion (GSH) to its oxidized form (GSSG) at the expense of hydrogen peroxide,
e) GR – Glutathion reductase. It reduces GSSG to GSH with concomitant oxidation of NADPH.

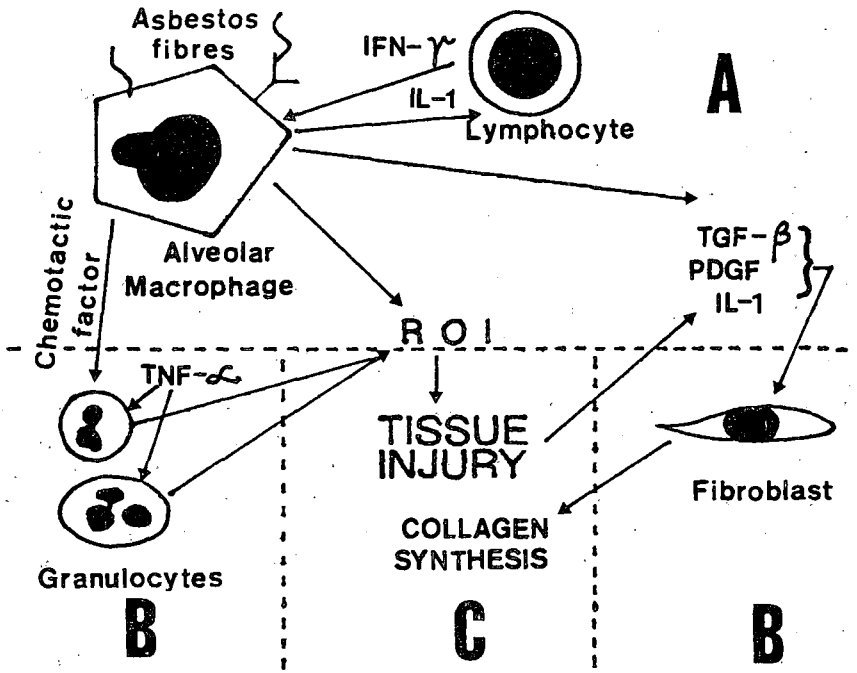

Fig. 2. Hypothetical mechanisms of cellular interactions leading to lung tissue fibrosis.
A. In the first stage, macrophages which do not phagocyte completely long asbestos fibers, release in an uncontrolled way an increased level of proinflammatory mediators, radical oxygen intermediated (ROI) and fibroblast growth factors. Macrophages may be additionaly stimulated to release these factors by interferon (IFN) originating from activated lymphocytes or by endocytosis of antibodies that bound asbestos fibres. Lymphocytes in turn remain under influence of interleukin-1 (IL-1) released by macrophages.
B. In the second stage there is an increased accumulation of chemo-attracted granulocytes which stimulated by the tumour necrosis factor-α (TNF-α) release an increased level of ROI. There is also concomitant accumulation of fibroblasts which proliferate in response to a transforming growth factor-β), (TGF-β), and platelet derived growth factor (PDGF), IL-1 which results in increased collagen deposition.
C. A highly increased level of ROI, originating from macrophages and granulocytes, may cause lung tissue injury. Oxidative lung tissue injury may in turn additionally elevate release of IL-1 by macrophages and since then the proliferation of fibroblasts and collagen deposition.

interactions in asbestos dust fibrosis occurs between macrophages and fibroblasts, as in the case of silica, shown by Heppleston and Styles (12). Fibroblasts seem to be attracted to the place of injury by fibronectin released by macrophages. Once attracted, fibroblasts are under the influence of other cytokines released from AM, as IL-1 which stimulate them to proliferate (22, 28, 33). The process of IL-1 release under the influence of asbestos fibres was shown by Hartman et al. (11). Fibroblasts may proliferate as well as a result of action of platelet-derived growth factors (PDGF) or transforming growth factor beta (TGF-beta) released from activated macrophages. The latter two cytokines (PDGF, TGF-beta) may also induce fibrogenic responses by stimulating the production of connective tissue. Other cytokine, TNF, was found to act on fibroblasts in a concentration-dependant manner, at a low concentration. It stimulates fibroblast proliferation and at higher concentrations it blocks growth triggered by other cytokines ((5). The process of cellular interactions in lung fibrosis is complex and besides the one stated above, between macrophages and fibroblasts, includes interactions between macrophages and PMNL. PMNLs are attracted to the place of inflammation by chemo-attractants released from macrophages, however, they are not well--recognized. Once attracted, they are under influence of cytokines as in the case of fibroblasts. PMNL, however, will respond differently to each of these stimuli.

Macrophages as a source of many important biological modifiers are crucial cells for immunological regulatory mechanisms towards enhanced or suppressed response. This dual role of cells is nothing new and may depend on the organ, they exist in (21), but this may be also an effect of harmful activity of e.g. asbestos fibers.

CONCLUSIONS

The importance of macrophages in immunological processes especially in asbestos-related diseases is beyond any question. Therefore, their activity should be the matter of intensive studies. Macrophages should be seen in asbestos-related diseases as protective cells but also as one of the population of cells that may cause directly or indirectly undesired harmful effects. It is suggessted by Strieter et al. (26) that the physiological role of AM may temper the immunologic reaction in the lung as they are less sensitive to the immunomodulating action of some cytokines and in this way prevent the inflammatory reaction. The chronic action of asbestos fibres may, however, change the whole regulation mechanism of macrophage activity and cause the release of many pro-inflammatory mediators, which in consequence may be responsible for tissue injury, fibrosis and cancer. One of the major and most harmful factors of tissue injury mentioned in this report may be the excessive production of reactive oxygen intermediates released from macrophages and chemo-attracted polymorphonuclear leukocytes. Excessive production of ROI, expressing the response of the lung to toxic chemical insult, may cause further damage to cells, which in turn may result in irreversible or nearly irreversible

lesions which are deleterious to normal lung function. The most deleterious and, as yet, irreversible effect of lung tissue injury, caused by the harmful activity of asbestos dust, and excessive lung response is the initiation, promotion and progression of cancer.

Macrophages, as one of the population of effector cells in tumour-killing, are the subject of studies which try to establish an effective model of cancer therapy i.e. such a therapy that will leave no living cancer cell that will not further proliferate and metastasize (31). The hopes that we set on macrophages are still only hopes. However, they will never come true if we do not get enough knowledge about the activity of these cells from various view-points. One of these view-points could include the prospective studies of macrophage activity in patients suffering from asbestos-related diseases: a commonly known agent of cancer.

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