Differences between rat alveolar and interstitial macrophages 5 wk after quartz exposure

GÖRAN ZETTERBERG,1 ANNE JOHANSSON,2,3 JOACHIM LUNDAHL,4 MARGOT LUNDBORG,2 CARL MAGNUS SKÖLD, GÖRAN TORNLING,1 PER CAMNER,2 AND ANDERS EKLUND1

1Division of Respiratory Medicine, Department of Medicine, and 4Department of Clinical Immunology, Karolinska Hospital, S-171 76 Stockholm; 2Division of Inhalation Toxicology, Institute of Environmental Medicine, Karolinska Institute, S-171 77 Stockholm; and 3The Wenner-Gren Institute, University of Stockholm, S-106 91 Stockholm, Sweden

Zetterberg, Göran, Anne Johansson, Joachim Lundahl, Margot Lundborg, Carl Magnus Sköld, Göran Tornling, Per Camner, and Anders Eklund. Differences between rat alveolar and interstitial macrophages 5 wk after exposure. Am. J. Physiol. 274 (Lung Cell. Mol. Physiol. 18): L226–L234, 1998.—Macrophages play an essential role in pulmonary host defense. We investigated differences between rat alveolar (AM) and interstitial (IM) macrophages after exposure in vivo to quartz, an inducer of intensive alveolitis. Rats were exposed to 0.5 ml of saline without (n = 8) or with (n = 8) 10 mg of quartz by intratracheal instillation. In a third group (n = 8), 10 mg of surfactant were added to the quartz mixture. Five weeks later, AM were recovered by bronchoalveolar lavage and IM by mechanical fragmentation of the lung, followed by enzymatic treatment. Contamination of AM to the IM fraction was calculated to be 12–15%. After quartz exposure, the expression of major histocompatibility complex class Ia was increased in both AM and IM fractions. The receptor corresponding to human complement receptor 3 increased in AM after quartz exposure, and AM from quartz-exposed animals had a lower metabolic activation. Our findings indicate that IM are immunocompetent cells and that differences between AM and IM fractions occur upon quartz-induced inflammation. This response is not affected by addition of surfactant.

adhesion; bronchoalveolar lavage; metabolic activation; phagocytosis; receptor expression

PHAGOCYTOSIS IS AN important functional property of macrophages in host defense. Several other mechanisms, such as release of inflammatory mediators, antigen presentation (10), and expression of different membrane receptors (14, 17), are also involved. In humans, the ability of alveolar macrophages (AM) to dissolve poorly water-soluble inorganic particles is of importance (21), since humans have an extremely slow mechanical clearance from the alveolar region (25).

Quartz exposure is associated with an inflammatory cell response characterized by an alveolitis with recruitment of inflammatory cells, particularly neutrophils (5). The reaction may result in pulmonary fibrosis and impaired lung function (5). Quartz exposure induces a number of reactions, as production of reactive oxygen metabolites and release of proteolytic enzymes (3, 23) and, moreover, an impaired chemotactic response have been reported (7). It is also known that exposure to quartz will result in an increased phospholipid biosynthesis with an accumulation of phospholipids in the alveolar space and in alveolar type II cells (15). In previous in vitro studies, we have demonstrated that quartz induces a protease-dependent shedding of complement receptor 1 from activated granulocytes (18) and that surfactant inhibits this quartz-induced downregulation (35).

Pulmonary macrophages are located in different compartments, i.e., interstitial macrophages (IM) and AM. A transition of monocytes from bone marrow via the blood to the pulmonary interstitium and alveoli has been proposed (30), together with a local proliferation of the macrophages (4). The circulating mononuclear phagocytes (monocytes) may migrate to the interstitial tissue through a complex process including rolling, adhesion, and activation, expressing surface receptors that are involved in the process of transmigration (6). One fundamental subject of study is the origin and differentiation of the macrophages in the different compartments. The AM are available by bronchoalveolar lavage (BAL), but, due to their location, the IM have been more difficult to separate and study. A method with mechanical and enzymatic treatment has been developed for this purpose (13, 14, 27, 30, 31).

Besides different localizations, it is reasonable to assume that the AM and IM populations exert distinct functions that are altered upon exposure to inflammatory agents and that the intensity and nature of these events have an impact on the net outcome of the inflammatory response. In this study, we aimed to investigate rat AM and IM in a model of intense acute quartz-induced inflammation and to determine the functional (adhesion, metabolic activation, phagocytosis), morphological (electron microscopy), and phenotypical (receptor expression) characteristics of these two macrophage populations 5 wk after exposure. Moreover, we also investigated if addition of surfactant mitigated the inflammatory response induced by quartz. Our hypothesis was that the AM and IM populations undergo distinct and specific alterations in an acute quartz-induced inflammatory event.

MATERIALS AND METHODS

Preparation of quartz. The quartz was prepared from natural sand by sedimentation in water (kindly provided by Prof. Åke Swensson, Dept. of Occupational Medicine, Karolinska Hospital, Stockholm, Sweden). It was not freshly crushed and consisted of 98.3% crystalline quartz and 1.7% amorphous SiO₂. There were no endotoxins in the preparation. With the use of a projection light microscope (Visopan projection microscope, Reichert, Austria), the geometric mean of the...
particles was measured to be 1.2 \mu m, with a geometric standard deviation of 1.6. Quartz was resuspended in physiological saline at a concentration of 20 mg/ml, and, for the intratracheal instillation, 0.5 ml (10 mg quartz) was given.

Preparation of surfactant. Surfactant was prepared from minced pig lungs (28). The preparation (kindly provided by Dr. Tore Curstedt, Karolinska Hospital), which consisted of 98–99\% phospholipids (30–35\% dipalmitoyl phosphatidylcholine) and 1–2\% of the hydrophobic surfactant proteins B and C, was resuspended at a concentration of 80 mg/ml in physiological saline. For the intratracheal instillation, a surfactant dose of 0.125 ml (10 mg) was added to the quartz suspension.

Animals. Alveolar and interstitial cells were obtained from 24 male Sprague-Dawley rats (Charles River, Uppsala, Sweden) initially weighing \( \sim 200 \) g. The rats were kept in cages containing one or two animals. Food and water were unlimited. The study was approved by the Ethics Committee for Animal Experiments in Stockholm (North).

Intratracheal instillation. Animals were anesthetized by intraperitoneal injection (2.7 ml/kg) of a mixture of one part of a preparation with 10 mg/ml fluanisone and 0.2 mg/ml fentanyl (Janssen Research Foundation, Brussels, Belgium) and one part of 1 mg/ml midazolam (Hoffman-La Roche, Basel, Switzerland) to two parts of sterile water. An additional intraperitoneal injection was given after 5 min with 1.0 ml/kg of 0.5 mg/ml atropine (NM Pharma, Stockholm, Sweden) diluted in sterile water to 0.05 mg/ml. When sedated, a cannula was passed through the mouth via the larynx into the trachea with the rat fixed in a semupright position. The animals were exposed to 0.5 ml of physiological saline with (n = 8) or without (n = 8) 10 mg of quartz. An additional group (n = 8) was exposed to physiological saline and 10 mg of quartz as above with 0.125 ml (10 mg) of added surfactant.

Preparation of interstitial and alveolar cells. Rats were killed by an intraperitoneal injection of pentobarbital sodium 5 wk after quartz exposure. The interstitial cells were isolated according to the method of Holt et al. (13) as modified by Sjöström et al. (31). The pulmonary vascular bed was perfused with chilled phosphate-buffered saline (PBS) introduced through cannulation of the heart, with the aorta first being perfused with chilled phosphate-buffered saline (PBS) introduced through cannulation of the heart, and the respiratory system was filled with 3 ml of cold saline and PBS. A fresh 10 mg/ml solution of collagenase was added to the lungs and the perfusion was continued until the lungs were clearly white. The lungs and trachea were removed from the chest, and BAL was performed with PBS (without Ca\(^{2+}\) and Mg\(^{2+}\), pH 7.4, 37°C). The alveolar cells were collected, and lavage of the lungs was repeated 15–20 times until only occasional cells were present in the lavage fluid. Thereafter, the lung tissue (weight range 0.37–0.45 g) was sliced into small pieces and digested with collagenase (140 U/ml; Boehringer Mannheim, Mannheim, Germany) and deoxyribonuclease (50 U/ml; Boehringer Mannheim) for 90 min at 37°C in a shaking water bath. Lung tissue that remained intact in the solution was disrupted by repeated passages through a Pasteur pipette before filtration through a gauze and centrifugation (300 g, 20°C, 10 min) of the cell suspension. The AM recovered by lavage were enzyme treated in the same way as the lung tissue. To exclude the possibility that the enzyme treatment per se could influence the phenotype of the cells, we have previously compared the expression of cell surface receptors on enzyme-treated and untreated AM, resulting in proteolytic degradation that did not, to any greater extent, influence the receptor expression (14). The numbers of cells obtained from the lung tissue as well as from lavage fluid were counted in a Bürker hemocytometer.

Differential counts. A differential cell count was performed after preparation of centrifugal smears from 5 to \( 6 \times 10^4 \) alveolar or interstitial cells in a Cytospin 2 apparatus (Shandon, Runcorn, UK) at 500 revolutions/min for 3 min. Cells were stained with May-Grünwald Giemsa, and a field of 500 cells was counted.

Transmission electron microscopy. Alveolar and interstitial cell fractions were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, postfixed in 1% OsO\(_4\) in 0.1 M cacodylate buffer, dehydrated in a graded alcohol series, and embedded in agar 100 resin (Agar Scientific, Stansted, Essex, UK). Thin sections, contrasted with uranyl acetate and lead citrate, were examined in a Jeol 1010 transmission electron microscope (Jeol, Tokyo, Japan).

Phagocytic assay. Phagocytic activity of macrophages from the alveolar and interstitial fractions was studied using a modification of the method described by Hed (12) and Ohansson et al. (14). From suspensions of alveolar and interstitial cells in complete medium (GIBCO, Paisly, UK), 250 ml (10\(^6\) cells/ml) were added to glass slides with round-bottomed wells (Nova Kemi). Cells were allowed to attach to the glass by incubation for 30 min at 37°C with 5% CO\(_2\) in air and 80% relative humidity. The medium was changed, and 250 ml of complete medium with 5 \times 10\(^5\) fluorescein isothiocyanate-labeled, heat-killed Saccharomyces cerevisiae (baker’s yeast; Jästbolaget, Stockholm, Sweden) were added. After incubation for 15 min, the reaction was interrupted by placing the slides in ice-cold Ringer acetate solution (Pharmacia & Upjohn, Uppsala, Sweden). Extracellular free yeast particles were rinsed off, and the cells were stained with trypan blue (2 mg/ml) for 30 s. Because trypan blue does not enter viable cells, the ingested yeast particles were distinguished by their green fluorescence and the surface-attached particles by their staining with trypan blue. The number of ingested and attached yeast particles per macrophage was determined using a Zeiss fluorescence microscope. In each sample, 200 consecutive macrophages were scored.

Expression of cell surface receptors. Alveolar (0.5 \times 10\(^6\)) and interstitial (0.5 \times 10\(^6\)) cell fractions were washed (300 g, 4°C, 5 min) in 2 ml of cold 0.15 M PBS, pH 7.4, supplemented with 0.1 mM EDTA and 0.02% sodium azide (PBS-EDTA). The cell pellets were then resuspended in 100 ml of PBS-EDTA and incubated at 4°C for 30 min with appropriate dilutions of the following mouse monoclonal antibodies: OX-6, reactive with rat rat IgG; OX-19, a monospecific MHC class II antibody (corresponding to MHC class II in humans); OX-24, a monospecific MHC class II antibody (corresponding to MHC class II in humans); CD54, often called intercellular adhesion molecule-1 (ICAM-1); OX-42, reactive with a membrane glycoprotein of rat macrophages corresponding to human complement receptor 3 (CR3); CD11b/CD18 or Mac-1) that is important for adhesion of monocytes and granulocytes to endothelium and matrix components; and CD71, a transferrin receptor, related to cell proliferation and intracellular iron concentration.

All antibodies were purchased from Serotec (Oxford, UK). Irrelevant antibodies of the same isotype were used as controls. The cells were washed (300 g, 4°C, 5 min) one time in 2 ml of cold PBS-EDTA, resuspended in 500 ml of PBS-EDTA, and examined in a flow cytometer (Epics XL Cytometer; Coulter, Hialeah, FL). Calibration was performed daily with Standard-Brite (Coulter) to ensure the same fluorescence level in each experiment. The leukocytes could be detected and separated by different light-scattering properties. The AM and IM cell clusters were gated in a two-parameter scatterplot histogram as described earlier (14). The percentage of positive-labeled cells was scored, and the quantitative levels of each antigen, expressed as mean fluorescence intensity (MFI) units, was calculated after subtraction of the background level.

Adhesion assay. An adhesion assay was performed as described earlier (14). Briefly, culture wells (Corning Labora-
Mann-Whitney significances were calculated according to the nonparametric ranges (interquartile ranges in Figs. 3 and 4). Statistical methods. Results are presented as medians (%) with ranges in parentheses; n = 8 experiments. See RESULTS and DISCUSSION with regard to uncertainty of contamination of alveolar cells to the interstitial fraction. NS, not significant. *P < 0.001, †P < 0.05, and ‡P < 0.01 vs. interstitial fraction.

In the electron-microscopic examination, cells were characterized as IM according to the following criteria: the nucleus occupied a relatively large part of the cell profile and a prominent part of the chromatin existed as heterochromatin; the surface was undulating but without the thin protrusions evident in AM; mitochondrial profiles were spherical to ovoid and had relatively few cristae; and a few primary and secondary lysosomes were present. Phagolysosomes often contained cell debris, as these cells frequently had phagocytized dead cells (Figs. 1 and 2). To validate the relative fractions of macrophages and lymphocytes from digested lung tissue found in the light-microscopic examination, the fractions of these cells were also estimated by electron microscopy. In the controls, the medians and ranges for macrophages and lymphocytes from the interstitial fraction were 62 (46–79) and 24% (8–32), respectively, of the cell population. For the quartz-treated animals, the corresponding data were 60 (51–69) and 25% (16–32). Because the light-microscopic and electron-microscopic data agree fairly well, our classification in the light microscope should be reasonably valid, with possibly a small overestimation of the lymphocytes and underestimation of macrophages from the interstitial fraction.

In the AM fraction of the quartz-exposed rats, 53% (39–58) of the macrophage profiles contained quartoiphil and conversely reduced macrophage fractions in lavage fluid compared with controls. The interstitial leukocytes from quartz-exposed rats did not differ from the controls, except the eosinophils (Table 1).

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Controls</th>
<th>Quartz</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alveolar fraction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macrophages</td>
<td>12.2</td>
<td>69.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>3.7</td>
<td>4.4</td>
<td>NS</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>0.5</td>
<td>2.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0.1</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Basophils</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Interstitial fraction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macrophages</td>
<td>56.6</td>
<td>57.0</td>
<td>NS</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>31.9</td>
<td>34.1</td>
<td>NS</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>2.0</td>
<td>8.7</td>
<td>NS</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>2.4</td>
<td>1.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Basophils</td>
<td>0</td>
<td>0.5</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Differential counts for leukocytes from the alveolar and interstitial fractions measured by microscopy.
particles. Most of them were engorged with quartz. In the interstitial fraction, 6% (1–21) of the macrophage profiles contained quartz, and the majority of these cells were probably AM. In some small cells (0–12%) with the appearance of IM, one or two quartz particles could be noted. In macrophage profiles with few small quartz particles, these were located in lysosomes. Some AM (4–26%) were filled with vesicles containing surfactant structures, but there were no differences in the percentage of such AM between quartz-exposed and control rats.

Phagocytic assay. In controls, the macrophages from digested lung tissue had significantly lower numbers of attached and ingested particles per cell compared with AM (Table 2). Accumulated attachment (i.e., the sum of attached and ingested particles per cell) and total interaction (i.e., percentage of cells with attached or ingested particles) were lower for macrophages from interstitial than from alveolar fractions. For the ingested fraction (i.e., number of ingested particles per cell divided by accumulated attachment), no difference was recorded. There were no differences in phagocytosis between the quartz-exposed group and the controls.

Expression of cell surface receptors. The expression of the OX-6 receptor was significantly higher on macrophages from digested lung tissue compared with AM in both controls and quartz-exposed rats. Exposure to quartz in vivo significantly increased the receptor density (MFI) and percentage of positive-labeled cells in both AM and macrophages from the interstitial lung fraction (Fig. 3A). No significant differences were recorded in the study of CD54 receptor expression (Fig. 3B).

The MFI value for OX-42 in control rats was higher on macrophages from the interstitial fraction than on...
AM, but the number of positively labeled cells did not differ. An increase in both MFI and percentage of positive cells was obtained in quartz-exposed AM but not in macrophages from digested lung tissue (Fig. 3C).

The CD71 receptor expression for controls was significantly higher on AM than on macrophages from the interstitial lung fraction with regard to both MFI and percentage of positive cells. For quartz-exposed ani-

Fig. 3. Receptor expression measured by flow cytometry in alveolar macrophage (AM) and interstitial macrophage (IM) fraction with mean fluorescence intensity (MFI) and percent positive cells in quartz-exposed and control rats. A: OX-6; B: CD 54 (observe the scale); C: OX-42; D: CD71. Results are expressed as medians, interquartile boxes, and ranges; n = 8 experiments in A–C and 4 experiments in D. *P < 0.05, **P < 0.01, and ***P < 0.001.
mals, a similar pattern was obtained, although it was only significant for the number of positive cells. The MFI of macrophages from digested lung tissue from quartz-treated rats was elevated compared with controls (Fig. 3D).

Adhesion assay. AM adhered more than macrophages from digested lung tissue, with the difference being significant for adherence to vitronectin in controls and to fibronectin in quartz-exposed animals (Fig. 4).

Metabolic activation. Metabolic activation in AM was significantly higher than in macrophages from the interstitial lung fraction both for controls and quartz-exposed animals. For the latter group, the metabolic activation in resting cells and PMA-activated AM were significantly lower than in controls (Table 3).

Surfactant exposure. Results from rats exposed to a mixture of quartz and a porcine surfactant preparation did not significantly differ from results obtained from rats exposed to quartz only. Data for the surfactant-treated group are therefore not presented.

DISCUSSION

We have earlier reported on functional, morphological, and phenotypical differences between rat AM and IM (14). The intratracheal mode of quartz exposure is a nonphysiological way to induce an inflammatory response. As an experimental method, it has the advantage of being reproducible and providing a defined dose of quartz into the target organ (8). The present study, which was undertaken to analyze differences between the two macrophage populations in the early stages of quartz-induced inflammation, confirmed our previous findings. In addition, we determined that exposure to quartz had no influence on the phagocytic properties or on the adherence to vitronectin- or fibronectin-coated surfaces. Quartz exposure reduced the metabolic activity in AM. Furthermore, the expression of MHC molecules, reflecting the ability to present antigenic peptides, was increased in both cell populations, whereas the expression of CR3 was more pronounced only in AM and that of the transferrin receptor CD71 only in macrophages from digested lung tissue. Incubation of the cells with a surfactant preparation had no influence on the results.

Intratracheal exposure to quartz induces an early intense inflammation that may progress to a fibrosing process, resulting in severe damage of the lung structure and function (9). The different roles in these events

![Fig. 4. Percentage of AM and IM adhering to surfaces coated by vitronectin and fibronectin in quartz-exposed and control rats. Results are expressed as medians, interquartile boxes, and ranges; n = 8 experiments for quartz-exposed AM and IM and control AM and 7 experiments for control IM. *P < 0.05.](image)
Table 3. Metabolic activation in alveolar macrophages and macrophages from the interstitial fraction

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Controls</th>
<th>Quartz</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alveolar fraction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting cells</td>
<td>6.6t (4.9–9.7)</td>
<td>4.1t (4.9–9.7)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>PMA activated</td>
<td>206.5t (130.8–287.7)</td>
<td>102.6* (30.1–240.4)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>FMLP activated</td>
<td>30.0t (19.8–51.4)</td>
<td>28.4* (11.4–61.4)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Intersitial fraction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting cells</td>
<td>1.4 (0.6–5.0)</td>
<td>0.7 (0.2–2.1)</td>
<td>NS</td>
</tr>
<tr>
<td>PMA activated</td>
<td>19.7 (5.2–101.6)</td>
<td>18.1 (7.3–28.0)</td>
<td>NS</td>
</tr>
<tr>
<td>FLMP activated</td>
<td>8.2 (2.2–23.2)</td>
<td>5.4 (1.5–9.1)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Results are represented as mean fluorescence intensity and are expressed as medians with ranges in parentheses; n = 8 experiments (except for interstitial controls in which n = 7). See RESULTS and DISCUSSION with regard to uncertainty of contamination of alveolar macrophages to the interstitial fraction. *P < 0.001 and †P < 0.01 vs. interstitial fraction.

Displayed by AM and IM, reflecting inflammation in the alveoli and interstitium, respectively, are incompletely elucidated. In the present study, a higher percentage of macrophages was observed in BAL fluid than in digested lung tissue in which lymphocytes and neutrophils composed higher proportions of the cell count. After quartz instillation, a pronounced increase in the neutrophils was observed in lavage fluid, but no corresponding change could be noted among the interstitial cells, indicating that inflammation is initially most pronounced in the alveoli.

The morphological appearance of the alveolar and interstitial cells differed in agreement with earlier reports (14, 30). Thus IM were smaller and smoother, contained fewer lysosomes, and had more heterochromatin than AM. Quartz exposure did not change this pattern. However, it must be kept in mind that a substantial degree of contamination of AM to the interstitial fraction may occur. Kobzik et al. (16) estimated it to be ~20%. In our study, the contamination of AM in the interstitial fraction was calculated from the finding of quartz particles in ~50% of the cells in the AM fraction compared with ~6% of the cells in the interstitial fraction. Thus the contamination could be calculated to 12–15%. A small proportion of cells (1–12%) with the appearance of IM in the digested lung tissue contained a few quartz particles. These could have been free quartz particles phagocytized during the enzymetreatment when macrophages from the interstitial fraction were separated from the lung tissue or, alternatively, could be particles that had passed through the epithelial cells and then were phagocytized in vivo.

Exposure to quartz did not change the phagocytic capacity of either AM or macrophages from digested lung tissue. Overall, the ability to phagocytize fluorescein-labeled S. cerevisae was lower in the interstitial fraction than in AM, in concordance with earlier reports of the phagocytic function (14, 27). Macrophages from the interstitial fraction had a lower number of accumulated attached particles per cell, but the ingested fraction was equal in both cell populations. Our interpretation is that the smaller macrophages from the interstitial fraction attached fewer particles, although the process of ingestion was not impaired.

Five weeks after instillation of the quartz suspension into the trachea, AM and macrophages from the interstitial lung fraction displayed a similar tendency of decreased capacity to adhere to plastic surfaces coated with vitronectin and fibronectin compared with controls. Additionally, and in concordance with our previous report (14), AM showed a greater ability to adhere than macrophages from the interstitial fraction. The differences in adhesive characters and in the attachment of yeast particles to the two cell populations indicate that disparate adhesion properties may be a generalized phenomenon. However, it must be emphasized that our adhesion methods utilize different receptor pathways.

The metabolic activity, measured as intracellular hydrogen peroxide production, was reduced in AM after quartz exposure. However, it was still higher in AM than in macrophages from the interstitial fraction. This finding is in agreement with an earlier report by Prokhorova et al. (27), although a superoxide anion release technique was used in their study. The results also concord with an earlier study (24) in which quartz did not cause any significant superoxide anion production in human AM. In contrast, Nyberg and Klockars (23) determined in cultured blood monocytes that quartz increased the reactive oxygen metabolite production as measured by lucigenin-dependent chemiluminescence. One plausible explanation for this discrepancy may be that cultured blood monocytes differ from noncultured AM, as has been reported with regard to some cell surface receptors (26). A contamination from neutrophils in measurements of hydrogen peroxide generation is unlikely, since AM were well differentiated from the neutrophils by the flow cytometry gating.

The phenotypic receptor expressions on both AM and macrophages from digested lung tissue were influenced by quartz exposure in several ways. Because we have only studied the expression at a single point of time, we have no information on the dynamic nature of the processes. The expression of OX-6, reactive with a constant determinant of the MHC class Ia (corresponding to human class II; see Ref. 22), was higher in both types of cells after exposure. In agreement with previous reports (14, 17), the expression was higher on macrophages from the interstitial fraction than on AM. Inhaled antigens can, after being processed in macrophages, be presented as peptides to lymphocytes by MHC molecules (6). The findings implicate macrophages from the digested lung tissue function as immunocompetent cells and should not be regarded as just precursors of AM. However, other receptor interactions are also supposed to be essential for the outcome of the antigen presentation. The macrophage-lymphocyte in-
teraction may be further enhanced by the ICAM-1 (CD11b/CD18; see Ref. 29). CR3, regarded as a generalized adhesion molecule (20), mediates adherence of monocytes and granulocytes to endothelium and matrix components (33) and is involved in phagocytosis (2). Despite an increased surface expression of CR3 in AM, the adhesion capacity was unaffected. The explanation for this discrepancy is unknown but agrees with our previous report in which inability of CR3 blocking ligands to inhibit adhesion was demonstrated (19).

To analyze a receptor regarded as reflecting receptor-mediated endocytosis, the expression of the transferrin receptor CD71 was measured (34). It functions as a cell membrane transportation receptor that recirculates and mirrors cell proliferation and intracellular iron concentration. In unexposed and exposed animals, similar patterns were observed, with higher expression in AM than in macrophages from the interstitial fraction. This is consistent with other reports (Haslam et al. (11) and Johansson et al. (14)); however, Johansson et al. observed a more pronounced expression on AM in granulomatous diseases. After exposure to quartz, the intensity of CD71 antibody fluorescence became more pronounced in macrophages from the interstitial fraction than in controls, possibly reflecting cell proliferation.

In a previous study, we determined that surfactant did inhibit a quartz-induced down-regulation of complement receptor 1 on granulocytes in vitro (35). However, in the present study, no protective effect of surfactant could be noted, since addition of surfactant to quartz did not influence the outcome of any parameter after 5 wk. Comparable results have been reported in an in vivo study of rats (1). In that study, a suspension of quartz and a bovine pulmonary surfactant resulted in a reduced quartz-induced increase in inflammatory parameters (total protein of BAL fluid, \( \beta \)-glucuronidase activity, and neutrophil number) after 1 day, but 14 days after the instillation, the protective effects were lost (our experiments were performed after 5 wk). One explanation might be that there is no lasting protective effect due to rapid turnover of surfactant that reduces the previous excess. Support for this notion was that, with repetitive surfactant treatment of the quartz-exposed rats for 7 days, the elevations of inflammatory parameters (total protein of BAL fluid, \( \beta \)-glucuronidase activity) were reduced (1).

In conclusion, our findings are in agreement with earlier reports of the morphological, functional, and phenotypical differences between AM and macrophages from digested lung tissue. However, the problems with contamination of AM to the interstitial fraction have to be kept in mind. Furthermore, we demonstrated that, at an early stage of quartz-induced inflammation, the most prominent effects were evident in the alveoli (e.g., there was a more pronounced accumulation of neutrophils in the alveoli than in the interstitium). Additionally, after quartz exposure, the AM had a lower metabolic activation. There were also changed phenotypic expressions in both cell populations, with the most pronounced difference between the two cell types being an enhanced expression of the complement receptor on AM. The impact of these distinct and specific alterations in respective macrophage populations during the early stage of inflammation on later fibrosing phases of the process necessitates further investigation.

We are grateful for skillful technical assistance by Marie Hallgren and Per Sandgren. We thank Dr. R. A. Harris for critical reading of the manuscript and linguistic advice.

This study was supported by grants from the King Oscar II:s Jubilee Foundation, the Swedish Council for Work Life Research (No. 91–0221), the Swedish Heart–Lung Foundation, the Swedish Medical Research Council (Grant 16X-105), the Swedish Society of Medicine, and the Research Funds of the Karolinska Institute.

Address for reprint requests: G. Zetterberg, Thoracic Medicine, Karolinska Hospital, S-171 76 Stockholm, Sweden.

Received 25 June 1997; accepted in final form 17 October 1997.

REFERENCES


