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PNEUMOTOXICITY OF DUST FROM ALUMINUM FOUNDRY AND PURE ALUMINA: A COMPARATIVE STUDY OF MORPHOLOGY AND BIOMARKERS IN RATS

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Abstract
Objectives: The overall objective was to assess the role of aluminum dust and fumes in the aluminum foundry (Al-F) in generating local inflammation in the respiratory tract, which may lead to induction and elicitation of occupational asthma and fibrosis. To understand the underlying mechanisms of involving particles from foundry, a long-term study was performed on rats. **Materials and Methods:** Pure α-alumina (Al-P) or (Al-F) was intratracheally instillated to rats in doses of 20 mg suspended in 0.5 ml of saline. After 3, 6 and 9 months since instillation, the following biomarkers were assessed in lung tissues: Clara cell protein (CC16), hyaluronic acid (HA), total protein, metaloproteinases (MMP) in bronchoalveolar lavage fluid (BALF), and GSH-S-transferase (GST). Morphological study of lungs and cells in BALF sediment was also performed. **Results:** In the long-term study, Al-F dust induced marked changes in both epithelial cells and lung tissues, leading to important remodeling in collagen deposit and elastase fibres after 6 and 9 months. By contrast, the same dose of Al-P caused an increase in the number of polymorphonuclear leukocytes in the lung and fibrosis, but the latter was manifested by only slight signs. The lung BALF showed a decreasing level of Clara cell protein and a markedly increased expression of MMP-2 and MMP-9. These findings suggest that there is an upregulation of MMP and an increase in epithelial cell death and Clara cells proliferation, which may contribute to the respiratory symptoms through remodeling of airways and alveolar structures. **Conclusions:** In conclusion, it must be said that CC16 is the most sensitive biomarker. Decreasing levels of this biomarker in BALF was observed in an early phase (3 months PE) of our study with serum aluminum (Al-S) concentration not exceeding 30 $\mu g/L^{-1}$. Foundry dust causes marked irritation and inflammation in the rat lung. In occupational exposure it may therefore be active in the human lung, and thus contribute to the chronic obturative pulmonary disease (COPD).

Key words:
Aluminum, Foundry dust, Clara cell protein, Metaloproteinases, Morphology

INTRODUCTION

Exposure to dust and fumes from the aluminum foundry (Al-F) may play a role in decreasing pulmonary function parameters and developing occupational asthma and fibrosis [1–4]. Significant respiratory-tract exposure to insoluble aluminum compounds, such as alumina (aluminum oxide, Al_2O_3) and soluble part of condensed aerosols occurs in the foundry environment [5].

The respiratory bronchiole and alveolar epithelium is a primary target site for inhaled agents that cause lung

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injury. Nonciliated Clara cells play an important role in the response during repair of the bronchiolar epithelium [6]. Clara cells cytochrome P-450 may catalyze xenobiotic biotransformation resulting in injury of these cells [7–9]. In distal airways, Clara cells serve as progenitors of bronchiolar epithelial cells [10,11]. Recently, Clara cell protein (CC16) in serum was proposed as a cell-specific marker for nonciliated bronchiolar epithelial cells, which play a role in immunomodulatory and/or anti-inflammatory processes [12–15]. In our recent studies it has been shown that CC16 is a sensitive biomarker of respiratory epithelial injury in exposure to welding fumes, glutaraldehyde, foundry aluminum, nitric oxides or metaloorganic compounds [16–21]. Clara cell proteins are involved in the pathogenic mechanisms, leading to fibrosis [13,22], chronic obturative pulmonary diseases (COPD) [23,24], and asthma in humans [4,25] as well as in the murine asthma model [26,27]. There are only a few data published from animal long-term studies of biological effects of exposure to different forms of alumina [28,29].

In our preliminary report it was shown that exposure to aluminum dust led to lowering levels of CC16 in bronchioalveolar lavage fluid (BALF) in rats [18]. Some of the BALF components, such as fibronectin, hyaluronan and collagen, were elevated in interstitial lung diseases [30,31]. Hyaluronic acid (HA) is synthesized in early phase of inflammation process primarily in fibroblasts, but it is also an indicator of connective tissue regeneration. We demonstrated that in rats exposed to glutaraldehyde, the ratio between CC16 and HA levels may predict fibrotic processes [17]. It should be noted that similar effects were demonstrated in animals after exposure to environmental dusts [32].

The aim of this study was to assess the role of aluminum foundry dust and fumes in generating local inflammation in the respiratory tract which may lead to induction and elicitation of occupational asthma and fibrosis. To this end, levels of different biomarkers: Clara cells protein, hyaluronic acid, extracellular matrix-degrading enzymes, and metalloproteinases (MMP) were determined along with the lung and BALF morphology.

MATERIALS AND METHODS

A group of 120 female Wistar rats of 250-280 g body weight (b.w.) were used in the experiment. All animals were kept in propylene plastic cages at a room temperature with a 12h light-dark cycle. In the present study, changes in BALF biomarkers were evaluated 3, 6 and 9 months after intratracheal instillation of alumina or foundry dust to rats in doses of 20 mg suspended in 0.5 ml of saline and compared with the results of the control group.

The animals were sacrificed by intraperitoneal administration of 50 mg/kg b.w. pentobarbital solution after 3, 6 and 9 months post exposure (PE). The trachea was cannulated when respiration had ceased and bronchoalveolar lavage was performed. The lungs were lavaged with normal saline using a total volume of 10 ml. BALF was centrifuged (200 g, 10 min, 4°C) and the cell-free supernatant was used for biochemistry. In the sediment, total number of cells and cell typing was assessed.

Dust analysis

In this study two types of dust were intratracheally instillated to rats; pure α-alumina, (Sigma-Aldrich, Germany) and condensed aerosols/dust collected on Staplex pomp in the foundry department. Before instillation dust samples were sieved through 36 μm ∅ strainer.

Identification of mineral composition of collected samples of foundry dust was performed with roentgen diffraction methods (XRD) on an x-ray Diffractometer D5005 (Siemens, Germany) with beam series CuKα (λ = 1.54056 Å). For identification of crystals phase computer basis ICPDS Powder Diffraction File was used. Scanning x-ray procedure reveals the presence of aluminum, alumina, silica and some part of crystals of Copper Sulfate ($CuSO_4$), Silicon Chloride ($SiCl_4$), Hieratite (K_2SiF_6), and Leightonite ($K_2Ca_2Cu(SO_4)_4 2H_2O$).

Analysis of hydrocarbons absorbed on surfaces of dust particles was performed on a gas chromatograph Agilent-6890, with an electronic pressure programer and split/splitless injector, equipped with autosampler. The hexane and acetone eluates of dust was separated on a DB-5MS capillary column (length, 30 m, ID, 0.25 mm, film thickness

0.25 µm). The detection was carried out in a mass spectrometer detector Agilent-5973 MSD-EI. Constituents of foundry dust were characterized qualitatively: polycyclic aromatic hydrocarbons that predominated, anthracene, phenanthrene 178, through benzo[a]pyrene 252-amu to dibenzo[a,h]anthracene amu-278-amu and phtalans 149-amu.

Aluminum determination
Blood samples were collected in Vacutainer (Bencton-Dickinson) for trace elements analysis and immediately placed at +4°C. Then samples were divided into two portions and stored at –20°C. Aluminum in serum was determined by graphite furnace atomic absorption spectrophotometer using Hitachi model Z-8270 Polarized Zeeman. Aluminum in serum samples was determined after deproteinization with water and 70% nitric acid. The matrix-based calibration was used for the quantitation. The precision, coefficient of variation (CV), of the method for serum at 2.5–15 µg L^{-1} was 7.4%.

Biomarkers
Clara cell protein was determined by latex immunoassay [33]. Specific rabbit antibodies against CC16 and a standard for CC16, based on the purified protein, were obtained as described earlier [15]. CC16 and total protein concentration in BALF was determined. GSH-S-transferase (GST) (EC. 2.5.1.18) in postmitochondrial supernatants of rat lung was assessed with 1-chloro-2,4-dinitrobenzene (CDNB) [34]. Hyaluronan (hyaluronic acid) was measured in non-concentrated BALF by enzymatic-immunoassay (ELISA) kit, including hyaluronic acid binding protein (HABP) capture molecule, Chungai-test (Japan). Matrix metalloproteinase (MMP) activities were measured by gelatine zymography. P-aminophenylmercuric acetate activated MMP-9 or MMP-2 (Calbiochem, France Biochem, Meudon, France) was used as control. Gels for electrophoresis comprised 0.1% (m/v) gelatine and 10% (m/v) polyacrylamide. Zymography revealed proteolytic activity, which appeared as clear zones, demonstrating lysis of the gelatine in gels against the blue background of Coomassie stained gelatine [35]. Proteolytic activity areas were measured by automated image analysis (Vilbert-Lourmat, Marne La Vallée, France) with Bio-1D software.

Morphology
For morphological analysis in an electron microscope, the lungs were fixed by intratracheal infusion (20 cm of hydrostatic pressure) with 1%/0.5% glutaraldehyde/paraformaldehyde mixture in cacodylate buffer (pH = 7.4). Small lung pieces (fragments) were fixed by immersion in the same fixative solution and postfixed in 1% osmium tetraoxide (OsO_4). The material was dehydrated in ethanol series and embedded in epoxy resin (Poly-Bed 812). Semithin sections (1 µm) of the lungs were cut on an Ultrotome-III LKB with glass knives, stained with toluidine blue and examined in a light microscope. Ultrathin sections (70 nm), double stained with uranyl acetate and lead citrate, were evaluated using an electron transmission microscope (JEM 100-C).
Special attention was paid to the following findings:
- free dust particles in lumen of alveoli (without cellular response);
- dust particles surrounded by macrophages and/or granulocytes;
- a granuloma-like structures within the lung tissue;
- alveolar brionchiolization;
- collagen fibre;
- elastine fibre;
- quantity of Clara cells.

Statistical analysis
Data were expressed as means ± standard deviations. Analysis of variance was used to determine difference between groups. Spearman correlation test was employed. A level of statistical significance was established at a value of $p < 0.05$.

RESULTS

Statistically significant decrease in CC16 levels were observed after 3 month along with concomitant changes in total protein and HA, which were found also 6 and 9 months after intratracheal instillation PE of pure alumina

(Al-P) in doses of 20 mg/kg (Table 1). Lung weight, expressed as lung/100g/b.w. showed significant increase after 6 and 9 months since intratracheal administration of both dust samples. Slightly decreasing GST activity with time in lung tissue were also presented. Administration of Al-F dust to rats evoked changes in expression biomarkers similar, but less pronounced than those found after instillation of alumina (Table 2).

Figure 1 shows the comparison of changes in biomarkers: CC16, GST, total protein and HA in the percentage of adequate controls after instillation of Al-P or Al-F. Concentrations of CC16 in BALF, 3 months after intratracheal instillation of the dust, dropped to 77.7% of the control value independently of the installed aluminum form. After 6 months since instillation of alumina and Al-F dust, the level of CC16 was 68.3% and 67.4% of control value, respectively. After 6 and 9 months, the HA levels in BALF were statistically higher in alumina treated rats than in controls. The Al-F instillation level of HA in BALF was higher in comparison with control, but whithout significant changes in time. This low HA level corresponds with the most pronounced changes in cytology and morphology of rats insulfated by foundry dust (Fig. 2) as compared to Al-P after 6 and 9 months PE (Fig. 3). After 3 months, the pulmonary tissue of rats insulfated with alumina was generally similar in its morphological characteristics to that of controls. However, focal accumulations of alveolar macrophages were observed within alveolar duct or pleura in some fragments of the lung. After 6 and 9 months, alumina induced numerous granuloma-like structures. After 3 months, Al-F dust produced in lungs similar changes to those observed after alumina instillation (Figs. 2 and 3). After 6 months, the influx of inflammatory cells in BALF (Figs. 2, 3, and 4) was seen and it was similar for both types of dust, however, that for alumina was mostly pronounced with significant increase in the percentage of neutrophils in BALF observed after 9 months (Fig. 3). After 3, 6 and 9 months since instillation of foundry dust, morphological changes in bronchioli and lung tissue were observed. In bronchiolar epithelium, the cilia of some cilliated cells were swollen. In some areas of the lung, bronchiolar epithelium lined the surface of alveoli (bronchiolisation) (Fig. 5). Six months after instillation of Al-F dust, interstitial infiltration consisted of young forms of lymphocytes, macrophages, and fibroblasts was

Table 1. Levels of biomarkers in BALF of rats (n = 10) 3, 6 and 9 months after intratracheal instillation of pure α-alumina (Al-P)

Time of examination PE	Lung/100 g b.w.		Total protein mg L^{-1}		CC16 mg L^{-1}		GST (nmol/CDNB GSH/min/mg protein)		HA $\mu g\ L^{-1}$	
	Exposure	Control	Exposure	Control	Exposure	Control	Exposure	Control	Exposure	Control
3 Months	0.7 ± 0.2	0.53 ± 0.01	252.4 ± 133.5	167.3 ± 104.7	7.8 ± 2.4*	13.2 ± 3.8	189 ± 9.8	191.9 ± 13	25.7 ± 16.5	18.7 ± 14.6
6 Months	1.5 ± 0.4*	0.98 ± 0.15	344.1 ± 165.1	204.1 ± 31.1	6.3 ± 3.9	9.9 ± 7.4	211.2 ± 30.7	225.7 ± 16.9	40.5 ± 17.3*	13.5 ± 7.4
9 Months	1.7 ± 1.1*	0.83 ± 0.15	384.8 ± 174.2	204.7 ± 30.9	7.5 ± 3.1	8.1 ± 2.0	222.6 ± 32.4	214 ± 23.6	52.8 ± 52.7	12.5 ± 7.1

* Statistically different from control, $p < 0.05$.

Table 2. Levels of biomarkers in BALF of rats (n = 10) 3, 6 and 9 months after intratracheal instillation of aluminum foundry (Al-F) dust

Time of examination PE	Lung/100 g b.w.		Total protein mg L^{-1}		CC16 mg L^{-1}		GST (nmol/CDNB GSH/min/mg protein)		HA $\mu g\ L^{-1}$	
	Exposure	Control	Exposure	Control	Exposure	Control	Exposure	Control	Exposure	Control
3 Months	1.0 ± 0.2	0.8 ± 0.05	242.5 ± 46.2	177.0 ± 101.0	8.9 ± 3.1	11.5 ± 4.1	242.5 ± 23.3	272.9 ± 28.5	26.1 ± 16.8	20.4 ± 28.5
6 Months	1.3 ± 0.3	0.87 ± 0.28	242.8 ± 94.2	219.8 ± 22.5	9.0 ± 3.2*	13.4 ± 1.6	218.2 ± 18.2	229.8 ± 22.5	30.3 ± 25.0	23.8 ± 11.4
9 Months	1.8 ± 0.7*	0.89 ± 0.21	497.6 ± 197.8	288.0 ± 74.7	5.3 ± 2.1	8.4 ± 0.9	201.3 ± 27.0	218.6 ± 9.6	34.6 ± 39.1	24.8 ± 17.5

* Statistically different from control, $p < 0.05$.

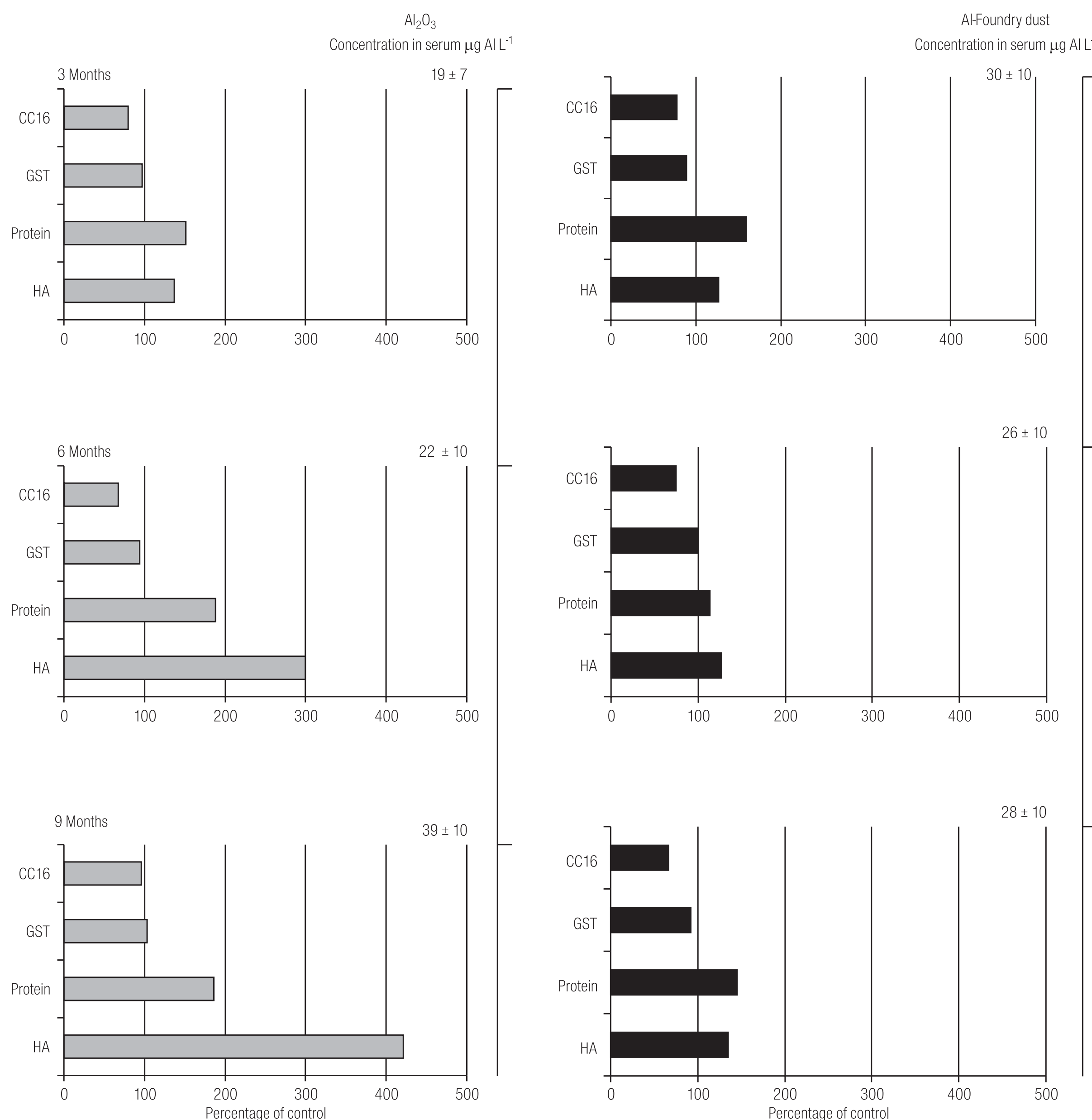

Fig. 1. Comparative study of biomarkers 3, 6 and 9 months PE intratracheal instillation of pure α-alumina (Al-P) or aluminum foundry (Al-F) dust in rats as a percentage of adequate controls.

Table 3. Correlation coefficients between neutrophils (%) and biomarkers in BALF after intratracheal instillation of pure α-alumina (Al-P) or aluminum foundry (Al-F) dust to rats

Dust	Correlation coefficients (p*)	CC16 mg L^{-1}	HA µg L^{-1}	GST (nmol/CDNB GSH/min/mg protein)	MMP-2 µg L^{-1}	MMP-9 µg L^{-1}	Lung/100g b.w.
Al-P	Neutrophils (%)	-0.27 (0.07)	0.31 (0.01)	0.46 (0.00)	-0.67 (0.00)	-0.64 (0.00)	0.62 (0.00)
Al-F		-0.36 (0.05)	–	-0.55 (0.00)	0.38 (0.04)	0.33 (0.07)	0.65 (0.00)

* Probability in Spearman's test.

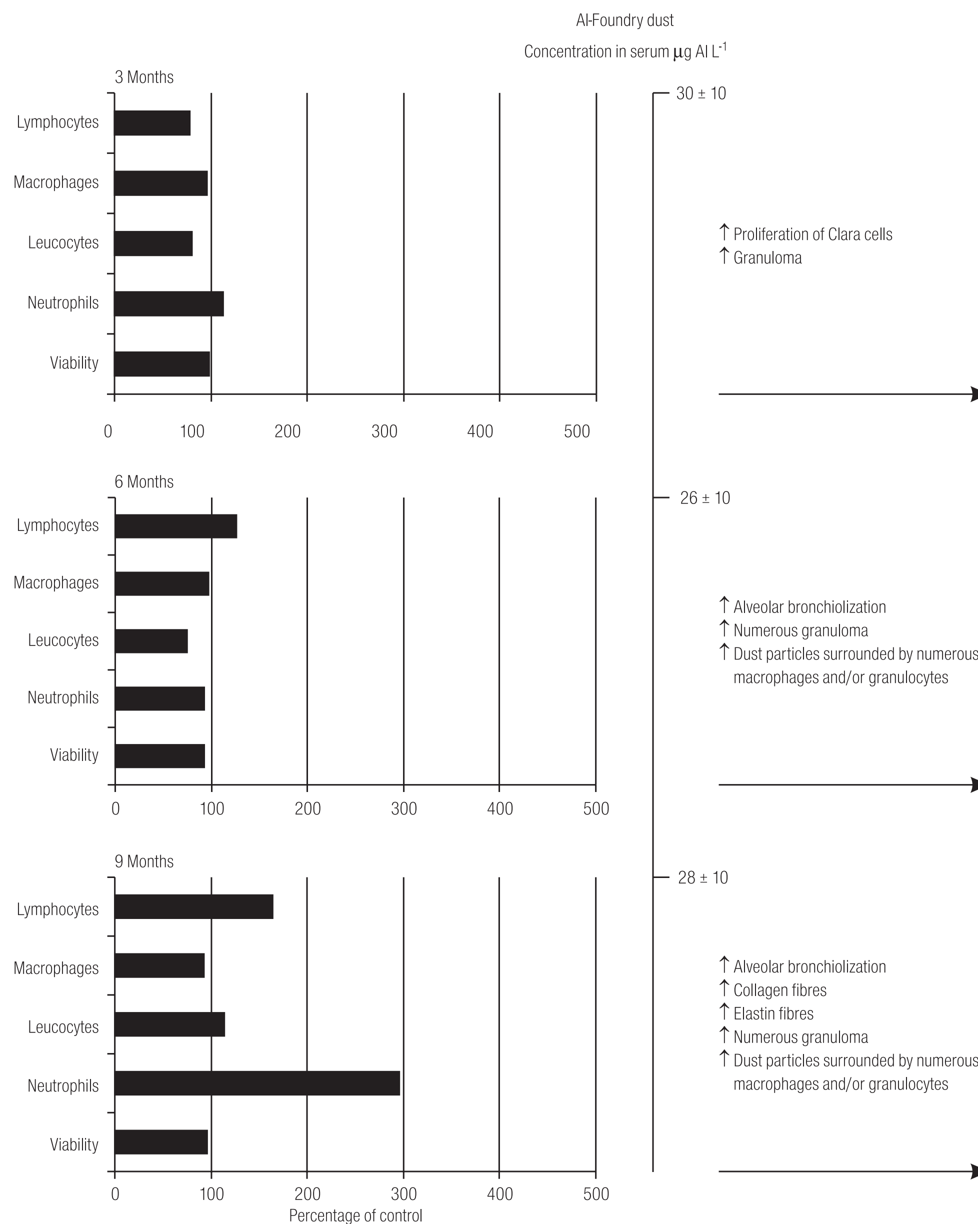

Fig. 2. Influxes of inflammatory cells in BALF and morphological dynamic changes 3, 6 and 9 months PE intratracheal instillation of aluminum foundry (Al-F) dust in rats as a percentage of adequate controls.

observed in peribronchial regions and within alveoli walls (Fig. 5). Interstitial fibrosis was more pronounced after 9 months. Numerous collagen and elastic fibres were observed within the alveoli walls (Figs. 6, 7, and 8). A significant increase in the thickness of basement membrane after 9 months was also shown (Fig. 9).

The negative correlation between CC16 and total protein, and the percentage of lymphocytes and neutrophils 3 months after Al-F dust PE are presented in Fig. 10. Aluminum in serum (Al-S) positively correlated with serum CC16 levels (in controls). The positive correlation between MMP-2 and neutrophils ($R^2 = 0.580$) was seen 6 months after Al-F dust instillation. The increased expresion of MMPs, 9 months after intratracheal instillation of Al-F dust, correlated with alveolar brionchiolization observed in the microscopic study after 6 and 9 months (Figs. 5 and 7). Table 3 shows the coefficients of correlation between neutrophil and study biomarkers. The difference in correlation slope in response to alumina or foundry dust were obserwed for GST and MMPs.

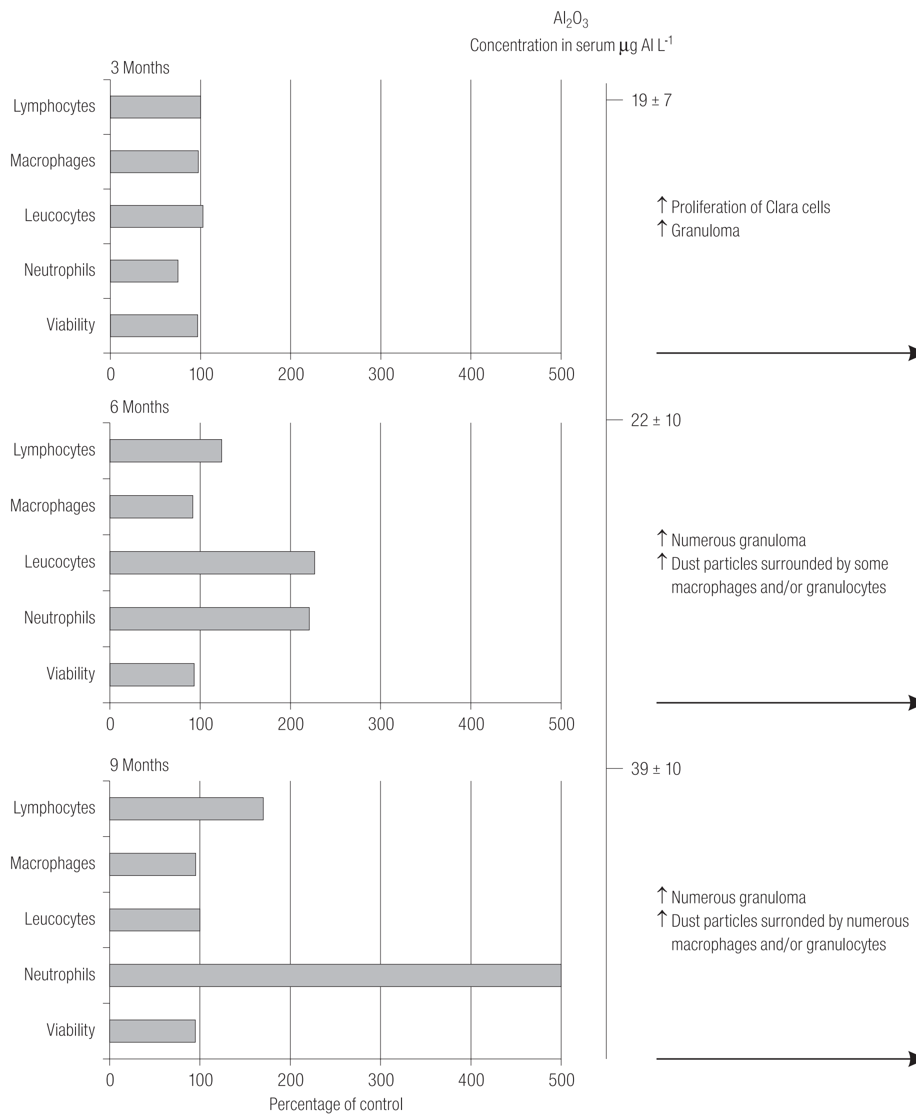

Fig. 3. Influxes of inflammatory cells in BALF and morphological dynamic changes 3, 6 and 9 months PE intratracheal instillation of pure α-alumina (Al-P) in rats as a percentage of adequate controls.

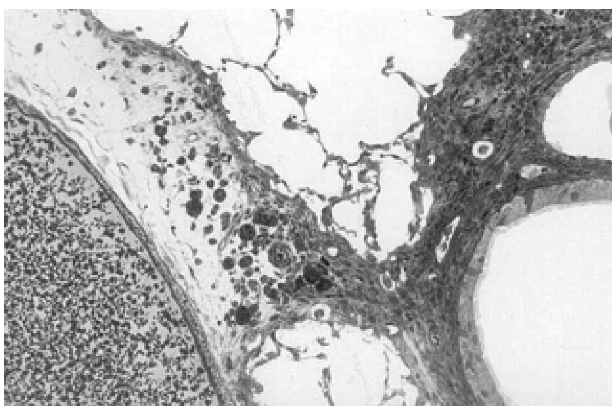

Fig. 4. The rat lung 6 months after instillation of aluminum foundry (Al-F) dust. A number of macrophages in perivascular spacium contained in cytoplasm dust particles are seen. Semithin epon section stained with tolluidyne blue (x360).

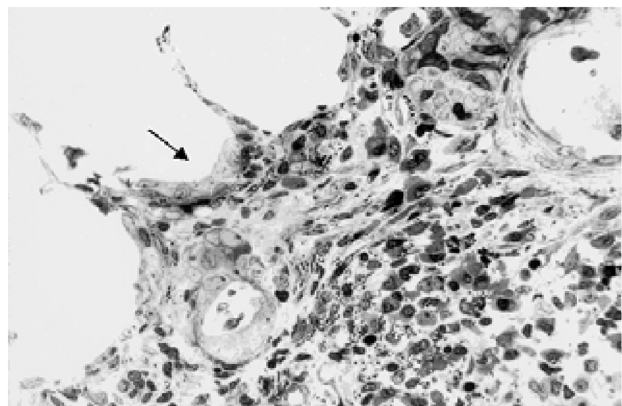

Fig. 5. The rat lung 6 months after instillation of aluminium foundry (Al-F) dust. Note proliferation of peribronchial lymphatic tissue with a number of fibroblasts and macrophages. Bronchiolization of lung alveoli is seen (arrow). Semithin epon section stained with tolluidyne blue (x360).

Fig. 6. The rat lung 9 months after instillation of aluminum foundry (Al-F) dust. Proliferation of collagen and elastin fibres is seen in the wall of the lung alveoli. Electron micrograph (x16000).

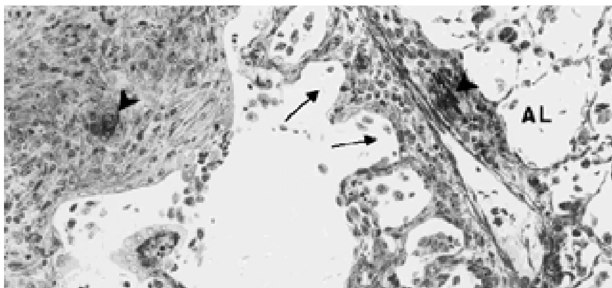

Fig. 7. The rat lung 9 months after instillation of aluminum foundry (Al-F) dust. Arrowhead shown conglomerate of alumina deposits surrounded by macrophages and fibroblasts. A number of macrophages in perivascular spatium contained in cytoplasm dust particles are seen. Note bronchiolization of lung alveoli (arrows). Semithin epon section stained with tolluidyne blue (x360).

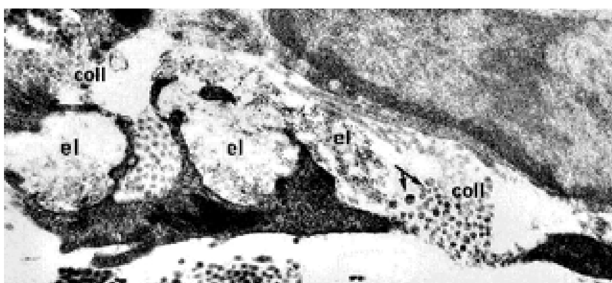

Fig. 8. The bronchioli wall 9 months after instillation of dust aluminium foundry (Al-F). Foci of proliferation of collagen (coll) and elastin (el) fibres are seen. Electron micrograph (x26000).

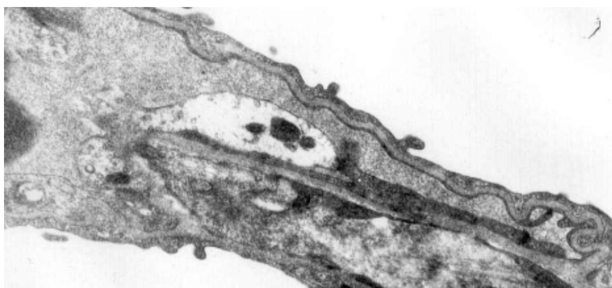

Fig. 9. The lung alveoli wall 9 months after instillation of aluminum foundry (Al-F) dust. Note the increased thickness of the alveoli basal membrane. Electron micrograph (x16600).

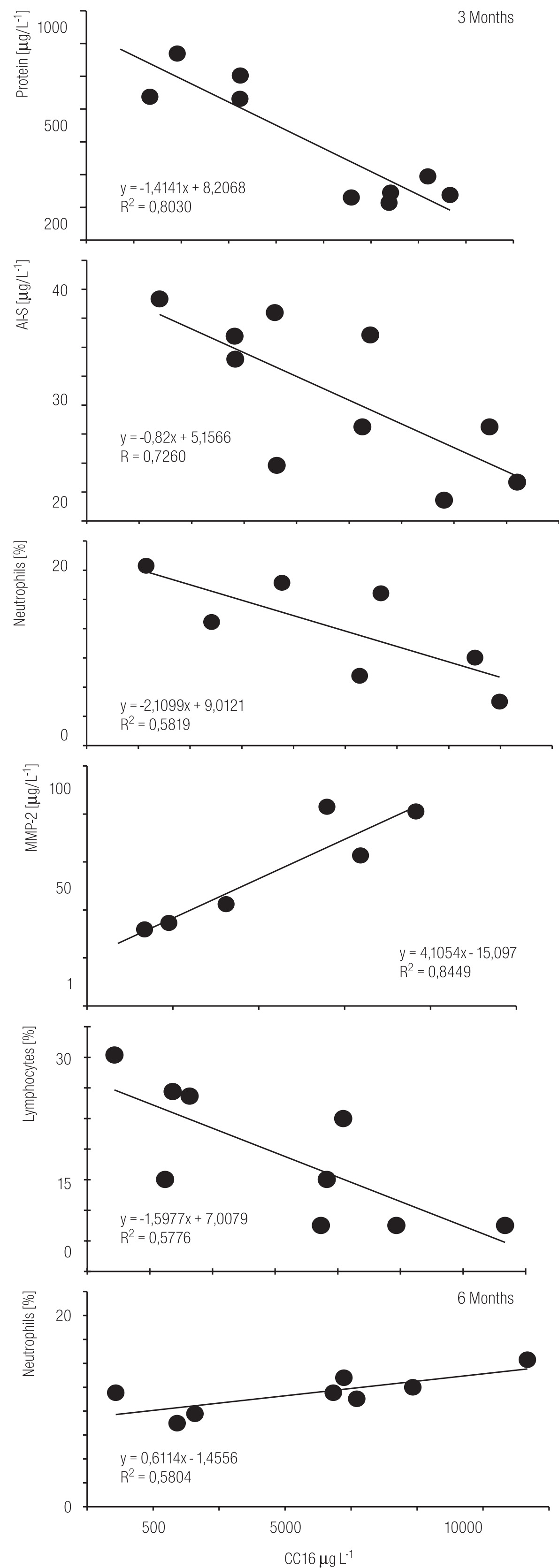

Fig. 10. Correlation between CC16 level in BALF and different markers after 3 months PE and between CC16 and neutrophils after 6 months PE instillation of Al-F dust in rats; $p < 0.05$.

DISCUSSION AND CONCLUSION

In our long-term study on rats considerable differences were found in investigated biomarkers and lung morphology after intratracheal instillation of Al-P and Al-F dust. Diminishing levels of CC16 in BALF after intratracheal instillation of Al-P and Al-F dust may correspond with the first inflammatory phase changes in airways (Tables 1 and 2, Fig. 1). In an early phase, interstitial infiltrations composed of young lymphocytes, macrophages and fibroblast as well as enhanced activity of MMPs were observed. The most characteristic influx of inflammatory cells, i.e. polymorphonuclear leukocytes were found after 6 and 9 months, concomitantly with high concentration of HA especially after alumina instillation (Fig. 2). Only slight signs of fibrosis were noted (Fig. 3). Significant negative correlation between the Clara cell protein levels and total protein in BALF (marker of blood/lung permeability), and between lymphocytes and neutrophils after 3 months PE confirmed anti-inflammatory action of CC16 related to Al-S concentration (Fig. 10). Lower quantity of Clara cells and diminishing levels of protease inhibitors secreted by Clara cells may lead to weak anti-inflammatory effects. The negative correlation between CC16 and Al-S were observed in control animals (Fig. 10). We also found the increased expression of MMP-2 and MMP-9, enzymes that degrade type IV collagen and elastin, major structural components of the basement membrane. This observation was similar to that made in acute stages of pulmonary inflammation in animal studies [36]. Matrix-degrading MMP enzymes, like free radicals, are not only directly responsible for airway and pulmonary injury and inflammation, but they also play an important role in the repair process [37–39]. It is likely that CC16 secreted from Clara cells may inhibit the production of MMPs from bronchial epithelial cells [40]. Expression of these MMPs is also increased in various types of inflammatory lung diseases in humans [41]. Finkelstein et al. [6], in their study on early changes in proinflammatory and profibrotic cytokine and antioxidant genes expression in lung cells, found an increase in mRNA and protein for interleukins, IL-1β, IL-6, and tumor necrosis factor (TNF-α). These changes are accompanied by changes in specific epithelial genes responsible for surfactant protein C and Clara cell secretory protein synthesis. It was shown that TNF-α, a proinflammatory cytokine, might induce the production of a counter-regulatory protein CC16 and thereby modulate airway inflammatory responses [42].

However, animals exposed to Al-F dust showed 6 and 9 months after instillation positive correlation (Table 3) between CC16 and MMPs, which supported an opinion that not only CC16, but also MMPs might be a factor in anti-inflammatory defence, acting through promoting proliferation of stem cells/Clara cells. MMPs facilitating migration of Clara cells (Figs. 5 and 7) and other bronchiolar cells into the regions of alveolar injury are the most important remodeling factor of the lung [5]. Foundry dust-induced lung injury, associated with transient up-regulation in terminal airway epithelium, compromised Clara cells which infuenced expression of MMPs and bronchiolization of alveoli in the second/remodeling phase processes 6 and 9 months after instillation (Figs. 5 and 7). Alumina, but not Al-F mobilized high quantity of hyaluronan low chains in BALF (Fig. 1). HA increased the accumulation of neutrophils in the lung and edema within the alveoli and lung interstitium (Table 3). Hyaluronan can activate fibroblast cytokines involved in pathological and physiological metabolic processes in connective tissue [43]. Low HA level in foundry dust-treated rats 9 month PE might accelerate lymphatic removal of fluid filtered across capillary walls (Fig. 1). Lung disorders were accompanied by increased wall permeability [44]. In foundry dust-treated rats, focal elastase and collagen fibres were found (Figs. 6 and 8). The elastase inhibitory capacities of various dust particles, including aluminum-silicate dust, may be of importance in the pathogenesis of industrial pneumoconiosis [45]. It was found, however, that mineral dust can induce airway wall fibrosis by direct upregulating proliferable and fibrogenic mediators as well as by matrix components in the airway epithelium and interstitium. Circulating inflammatory cells are not required for induction of these effects [46]. The results of the study indicate that Al-F dust, possibly because of ad-

hered substances, induces early changes in alveolar cell populations, including persistent neutrophilia. These cellular changes may have a destructive effect. The late pronounced increase in fibronectin in both Al-P and more extensively in Al-F dust-exposed rats indicates a delayed effect of alumina on the extracellular matrix. Intensive bronchiolization indicates the occurrence of compensation processes concomitant with the participation of enzymes involved in biotransformation of chemicals, mostly of organic nature, absorbed on the surface of small parts of dust. Detoriation of metabolic activity in the lung after instillation of Al-F dust may reflect decreasing activity of GST (Tables 2 and 3). In the lung, GST protects Clara cells from exogenous insult caused by bioactivated toxicants [47]. The appearance and disappearance of the lesion in the Clara cell correlated well with the activity of cytochrome P-450 monooxygenase in the Clara cell [48,49]. Clara cells during differentiation are more susceptible to injury by environmental toxicants, and thus to failure in airway regeneration [50]. Many animal studies showed toxic effect of naphthalene on Clara cells after i.p. exposure [51,52]. Only a few studies used inhalation exposure to naphthalene. Injury of Clara cells was demonstrated in mice exposed by inhalation to naphtalene even in concentrations considerably below 10 ppm, standard TLV OSHA for humans [8]. Rats are considerably more resistant to naphthalene, which correlates with the low presence of isoform cytochrome P-450 monoxygenases, CYP2F in Clara cells [9]. Our results suggest that inflammatory repair response may play a crucial role in the development of fibrotic changes in rats. Inflammatory response and repair resulting in pathological transformation of lung in the rat model could be monitored by such biomarkers as CC16, HA and MMP-2, MMP-9.

In conclusion, it must be said that Al-F dust causes marked irritation and inflammation in the rat lung, and that CC16 is the most sensitive biomarker for this process. A lowering level of this biomarker was observed in the early phase (3 months PE) with serum Al-S concentration not exceeding 30 $\mu g/L^{-1}$ Foundry dust may therefore be active in human lung and thus contribute to the chronic obturative pulmonary disease (COPD).

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