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Overview of Pulmonary Alveolar Macrophage Renewal in Normal Rats and during Different Pathological Processes

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We report experimental results on pulmonary alveolar macrophage (PAM) renewal in healthy rats and in rats treated with particles introduced in the lungs. Morphometric studies showed that the lungs of normal rats of the strain used in our study contain 20×10^6 PAM, 50×10^6 monocytes in alveolar capillaries, and about 3×10^5 interstitial macrophages. Pulse labeling with a tritiated thymidine (^{3}HT) gave a labeling index of 0.4% for the monocytes, of which a few could be observed in mitosis within alveolar capillaries. These monocytes are likely to be the PAM precursors. The daily input (> 4%) by PAM proliferation exceeds PAM loss by migration to the upper respiratory tract (2.5%). The life span of PAM was measured by sequential counting of lavaged cells after labeling with [^{125}I]iododeoxyuridine instilled intratracheally. The pulmonary lavage procedure used allowed us to recover at least 80% of the whole PAM population. A daily loss of PAM of 8–9% was measured, of which loss by death in the endoalveolar compartment was estimated at 5–6%. During the pathological processes studied, several parameters of PAM renewal were shown to be modified. PAM migration to the upper respiratory tract was frequently inhibited, PAM cytotoxicity was observed, and PAM proliferation increased in some cases and decreased in others. Under most of the pathological conditions investigated, the renewal of endoalveolar macrophages appeared quite different from that in normal rats, and direct blood monocyte migration to the endoalveolar compartment became a major component of PAM renewal.

Introduction

Although some experimental studies have concluded that pulmonary alveolar macrophages (PAM) are derived from bone marrow monocytes (*1–4*) as are other tissue macrophages (*5*), the relative importance of monocyte influx and local production of PAM under normal conditions and during pathological processes is still debated. Local production of PAM can be due to PAM proliferation and/or to release from proliferative PAM precursors located in the interstitium (*6–8*). Quantitatively, the intraalveolar proliferation of PAM is not negligible; it produces several percent of new PAM per day (*9–12*). Some experimental results under steady-state conditions have shown that most PAM could be directly derived from actively dividing cells (*13*), but also that PAM do not correspond to a self-renewing population (*14*). Thus, we have formulated the hypothesis that PAM proliferation is a maturation process of new precursors migrating to the endoalveolar compartment (*15*). In fact, most parameters involved in the balanced renewal and loss of PAM for the maintenance of homeostasis in normal conditions or its evolution during pathologic processes have not been measured.

The aim of this work was to provide experimental results for a better understanding of PAM homeostasis under steady-state conditions and modifications of the endoalveolar cell population during pulmonary injury induced by inhaled or instilled particles. All these studies were performed in male SPF Sprague-Dawley rats.

Studies in Normal Rats

Quantitative Analysis of Lung Cells

Most PAM can be identified by standard light microscopy, but the identification of their expected precursors requires more elaborate histological methods. Thus, we have studied the cellular composition of alveolar tissue in 3-month-old rats using electron microscopy. Table 1 shows this cellular composition, expressed as the percentage of total cell sections containing an observable nucleus. Because the first results showed a large number of leukocytes in alveolar capillaries from lungs fixed by intratracheal instillation of buffered glutaraldehyde without previous animal bleeding (group I), two other groups were studied. In group II, animals were bled before lung fixation, and in group III, animals were killed under anaesthesia by an intravenous injection of a saturated solution of ammonium sulfate producing an instantaneous cardiac arrest. No significant difference in the intracapillary cell composition was observed among the different animal groups. From these values we concluded that leukocytes are normally present in alveolar capillaries and that intracapillary monocytes are much more numerous than PAM. The number of interstitial cells, morphologically distinct from fibroblasts (mast cells, lymphocytes,

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Table 1. Composition of alveolar tissue as percentage of total cell sections with a nucleus.

Cell	Group[a] I	II	III	Mean	SD
Endoalveolar	37.8	39.5	39.1	38.9	1.1
Fibroblast	22.0	20.9	23.6	22.2	1.3
Type I cell	9.2	7.7	8.0	8.1	0.8
Type II cell	12.3	10.7	10.7	11.0	0.9
Type III cell	1.0	0.9	1.3	1.1	0.2
Macrophage	1.6	2.5	1.5	1.9	0.6
Monocyte	6.3	5.8	4.1	5.2	1.1
Lymphocyte	9.1	9.9	8.6	9.2	0.6
Polymorphonuclear	1.1	2.2	3.0	2.2	0.9
Number of observed nuclei	2018	1962	1164		

[a]I, not bled; II, bled; III, cardiac arrest.

poorly differentiated cells, etc.) was very low and was not measured systematically in all groups. These cells were quantified in groups of animals at 3, 12, and 18 months of age, corresponding respectively to 0.2, 1.6, and 2.5% of cell sections with an observable nucleus (*16*). Most of the cells (>90%) were mastocytes or lymphocytes. This significant increase in interstitial cells was the only change in the cellular composition of alveolar tissue related to aging.

A morphometric analysis was designed to determine the actual cell composition of lungs. The Delesse's equation was applied to each cell type:

$$N \times Vu = n \times S$$

where N is the number of nuclei per unit of tissue volume; Vu is the volume of the nucleus; n is the number of nuclei sectioned per unit of tissue area; and S is the mean area of sectioned nuclei.

The nuclear volumes were estimated from serial 0.5-μm sections. These volumes were 51.3 $\pm$ 3.7 (SD), 77.6 $\pm$ 10.9, 95.1 $\pm$ 6.6, 73.3 $\pm$ 9.3, 97.2 $\pm$ 13.8, 57.6 $\pm$ 14.8, 48.4 $\pm$ 4.5, and 34.3 $\pm$ 3.8 μm for endothelial cells, fibroblasts, type I epithelial cells, type II epithelial cells, PAM, monocytes, lymphocytes, and polymorphonuclear cells, respectively. The value of n was measured from the total number of cell nuclei in the semi-thin section and the cell composition (Table 1). The value of S was measured by electron microscopy based on more than 2000 nuclear sections. Table 2 shows the actual composition of alveolar tissue. Capillary leukocytes made up about 20% of all alveolar cells. PAM and monocytes represent 2 and 5%, respectively of the alveolar cells. Compared to others (*17*), this study gives a similar cell composition for alveolar tissue when capillary leukocytes are excluded.

The total number of cells was measured after morphometric analysis or DNA dosage. The mean volume of rat lungs was 10.58 $\pm$ 0.24 mL. Alveolar tissue corresponds to 89.3 $\pm$ 0.4% of total lung volume. The volume of nuclei was 0.78 $\pm$ 0.11% of the total volume of alveolar tissue. From these values, the total alveolar cell population was estimated to be 1160 $\times$ 10^6 cells. We measured the area of bronchial and bronchiolar epithelium. Because 15.2 $\pm$ 1.6 epithelial cells were observed on semi-thin sections per 100 μm of the epithelium, the total number of bronchial and bronchiolar epithelial cells was less than 10 $\times$ 10^6. Assuming that the other extra-alveolar populations corresponded to 20 $\times$ 10^6, the total number of lung cells

Table 2. Composition of alveolar tissue as percentage of total alveolar cells.

Cell	Percent
Endoalveolar	39.4
Fibroblast	21.1
Type I cell	6.3
Type II cell	11.7
Type III cell	1.2
Macrophage	2.1
Monocyte	5.2
Lymphocyte	10.8
Polymorphonuclear	2.3

Table 3. Leukocytes in blood and alveolar capillaries.[a]

Cell	Blood	Lungs
Polymorphonuclear	24.2 $\pm$ 2.1	25.3 $\pm$ 10.3
Lymphocyte	163.5 $\pm$ 3.9	118.8 $\pm$ 7.7
Monocyte	12.7 $\pm$ 2.9	52.2 $\pm$ 12.1
Total	200	200

[a]Mean values $\times 10^6 \pm$ SD.

was about 1190 $\times$ 10^6. DNA dosage, according to Burton (*18*), showed that the whole lungs contained 9.38 $\pm$ 0.77 mg of DNA. Because 10^6 PAM extracted by pulmonary lavage contained 9.16 $\pm$ 0.35 μg of DNA, we estimated that lungs contained 1023 $\times$ 10^6 cells. This value is in reasonable accord with that measured by morphometric methods. Macrophages located in the walls of large blood vessels, bronchi, and bronchioles corresponded to about 1% of all extra-alveolar cells, i.e., 3 $\times$ 10^5 cells in the normal rat. As these macrophages represent only 1 to 2% of the total PAM, it is unlikely that they could contribute significantly in number to macrophages harvested from mechanically disrupted lung tissue (*13*). Table 3 shows the numbers of leukocytes in alveolar capillaries and in the whole circulating blood. These two compartments contained a similar number of leukocytes but, relatively, monocytes were five times more numerous in lungs than in whole circulating blood (*20*).

Histological Study of Proliferating Lung Cells

The renewal of cells in alveolar tissue was studied after repeated intramuscular injections of tritiated thymidine (^{3}HT, 10 TBq/mM, 37 KBq/g) at 12-hr intervals (*21*). Colchicine (1.5 μg/g) was administered by intraperitoneal injection 4 hr before animal killing. Table 4 shows the mitotic index (MI) and ^{3}HT labeling index (LI) observed by light microscopy. The global LI gradually increased as a function of time after the first ^{3}HT injection but concommitantly, a gradual decrease in MI was observed. During the first 24 hr, the LI of PAM showed a nearly linear increase from 2.1 to 9%. Thereafter, it gradually decreased to 4.5% at 48 hr. A decrease of type II epithelial cell LI was also observed at 48 hr as compared to 36 hr. These decreases showed the radiotoxicity of repeated ^{3}HT injections at the doses used. PAM appeared to be one of the most sensitive cell types, suggesting that labeled PAM were derived from continuously proliferating cells located outside the endoalveolar compartment. Electron microscopy showed that the continuous increase of global LI was due mainly to a gradual increase in the LI of capillary leukocytes up to 48 hr after the first ^{3}HT injection (Table 5). A few monocytes (0.4%) were labeled after the first injection, as were 1.8% of lymphocytes. The proliferative ability of monocytes was confirmed by the observation of monocyte

Table 4. Mitotic (MI) and labeling (LI) indexes after repeated [^{3}H]thymidine injections at 12-hr intervals.

	Percent[a]				
	+ 0 hr	+ 12 hr	+ 24 hr	+ 36 hr	+ 48 hr
LI	1.2	4.1	5.9	8.8	11.8
MI	0.35	ND	0.09	0.10	<0.02
Type II cell LI	1.0	1.5	2.0	3.2	2.0
Macrophage LI	2.1	4.6	9.0	7.6	4.5

ND, not detected.
[a]Mean values of two animals per time.

Table 5. Labeling indexes of capillary leukocytes after repeated [^{3}H]thymidine injections at 12-hr intervals.

	Labeling index, %			
Time	Monocytes	Lymphocytes	Neutrophils	Eosinophils
+ 0 hr	0.4	1.8	0.0	0.0
+ 24 hr	38.1	11.5	1.4	0.0
+ 48 hr	55.7	7.7	25.8	80.0

mitoses in capillaries. Lymphocyte mitoses were also observed. Except for measurements performed after a single ^{3}HT injection, monocyte LIs were the same as those reported for blood monocytes using a similar labeling procedure (*22*).

These results showed that some monocytes (about 2×10^5) are proliferative cells in the alveolar capillary compartment. This population in S phase is quantitatively equivalent to the whole interstitial macrophage population where only few labeled cells were identified at 4 hr after ^{3}HT labeling. From 24 hr after injection, about half of the monocytes were labeled. After that time, PAM LI decreased due to the radiotoxicity of the repeated ^{3}HT injections. Therefore, in the normal rat, PAM are not directly derived from blood monocytes. If blood monocytes are PAM precursors, they have to divide in lungs either before or just after their migration to the alveoli.

Pulmonary Lavage and Clearance of Particles by PAM

PAM are easily extracted from lungs by pulmonary lavage. However, one has to know what fraction of the total PAM population is recovered when parameters of the renewal are studied. This can be achieved by cytoplasmic labeling of PAM after the inhalation of poorly soluble, nontoxic particles. Figure 1 shows the lung clearance of ^{59}Fe after inhalation of radioactive hematite by conventionally bred or specific pathogen-free (SPF) rats (*23*). The curves can be fitted by two exponential function components. The daily clearance rate was about 2.5% (i.e., from 5–10 days to 100–130 days after the inhalation), and the cleared radioactivity was entirely recovered by gastro-esophagal catheterization. This amount was contained in about 5×10^5 cells collected daily. During that time, most PAM contained particles. Thus, in the normal rat, 2.5% of the PAM population is removed daily by migration to the upper respiratory tract (*15*). During this period, the ratio of extracted particles to the total lung burden corresponded to the recovery yield of PAM by pulmonary lavage, as only few particles were sequestered in the interstitium (*24*). The recovery yields, measured at 12 and 37 days after hematite inhalation, were 88.0 ± 1.4 and 85.2 ± 4.4%, respectively. These values were confirmed using histological methods. The large recoveries observed [twice the previous reported value

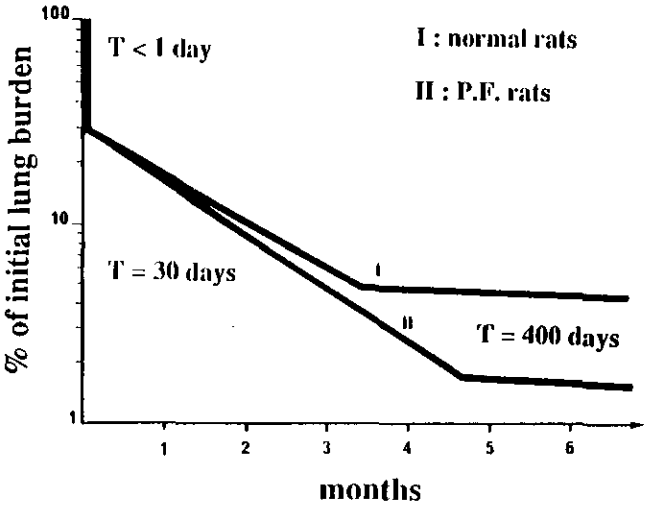

FIGURE 1. Lung clearance of radioactive hematite by conventionally bred or specific pathogen-free rats.

(*24*)] were due to a modification of the usual lavage procedure: lungs were kept for a few minutes under slight vacuum to collapse most alveoli before the first instillation of saline.

Proliferation of PAM

Intravenously administered ^{3}HT sometimes failed to be incorporated into PAM, whereas a reproducible incorporation of this label was obtained after lung incubation *in vitro* (*9*). This was the usual procedure we used to measure the proportion of the PAM population synthesizing DNA. In the normal rat, the mitotic index is about 0.08% and the LI 2.5 ± 0.5%. Thus, the local production of PAM could be calculated from the duration of the S-phase. Values at 5.8 hr (*25*) or 8 hr (*26*) have been reported. After intratracheal instillation of [^{125}I]5-iodo-2-deoxyuridine (IUdR), a single wave of PAM mitoses was observed, and we estimated the mean length of S phase at about 12 hr (*15*).

To prevent cell death by irradiation, ^{3}HT was administered at lower dose by a single intravenous injection. Under these experimental conditions, all the labeled cells could not be visualized by autohistoradiography. At 1 and 2 days after injection, 10–20% of PAM in mitosis were labeled, whereas no labeled mitosis was observed after 3 days. These results confirmed that most PAM proliferation corresponded to a maturation process of the newly migrating precursors (*15*), and, therefore, the total daily production of PAM was estimated at about 8%.

The daily PAM production by local proliferation only (at least 4%) exceeds the daily loss of PAM (2.5%) by migration to the upper respiratory tract. Because PAM migration to the interstitial compartment appears to be negligible in the normal rat, only PAM death in the endoalveolar compartment could account for homeostasia. Local PAM death was indicated during our lung clearance study after hematite inhalation: from 5 to 10 days to 100 to 130 days, most PAM contained particles, although the number of particles had decreased by a factor of almost 10. Thus, particles appear to be redistributed in the PAM population by phagocytosis after their release in the endoalveolar compartment after PAM death and lysis.

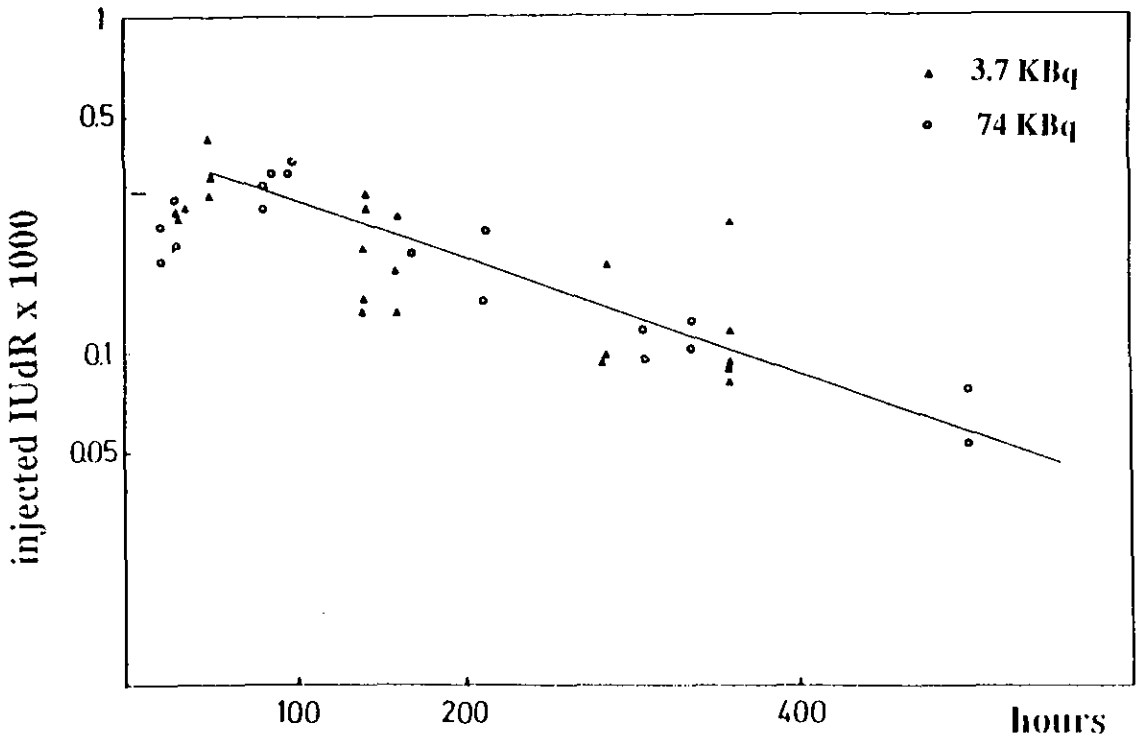

FIGURE 2. Amounts of ^{125}I in lavaged PAM as a function of time after $^{125}IUdR$ intratracheal injection.

Measurement of PAM Disappearance

With the hypothesis that S-phase macrophages could be adequate tracers of all intra-alveolar macrophages, we measured the disappearance rate of PAM labeled with $^{125}IUdR$ as a function of time after its intratracheal instillation. Local administration was performed to prevent labeling the expected PAM bone marrow precursors; $^{125}IUdR$ was used because it is reused far less after cell death than ^{3}HT (*27*). Figure 2 shows the amount of ^{125}I in PAM extracted by pulmonary lavage as a function of time after the intratracheal injection of $^{125}IUdR$ at two different doses (3.7 and 74 KBq). During the first hours after the injection, ^{125}I measurements showed some variability. Thus, we adjusted the values to an initial incorporation of IUdR of 0.3% of the amount instilled, which corresponds to the mean value observed for the two groups. Clearance of ^{125}I in PAM was not significantly different in the two groups. The clearance of ^{125}I in PAM after IUDR administration could be fitted by an exponential function with a half-life of about 9 days. No decrease was observed up to 2 days after instillation. This delay could be explained either by migration to the alveolar compartment of locally labeled precursors or by a special behavior of PAM within a few hours after their cell division. Autohistoradiographs performed after the 74 KBq administration showed a similar evolution of PAM LI.

The daily ^{125}I decrease here measured as about 8% is interpreted as the total daily loss of PAM if a spontaneous release of ^{125}I after incorporation of IUDR does not occur to a significant degree. Since PAM migration to the interstitium is negligible and daily PAM migration to the upper respiratory tract is 2.5%, the daily loss of PAM by death in the endoalveolar compartment is about 5 to 6%. The total loss has to be balanced by the daily production of PAM; our previous results from *in situ* input suggests that about 4% of precursors ought to enter the alveolar space daily and divide.

Studies during Different Pathological Processes

Intratracheal Instillation of Beryllium and Aluminum Hydroxides

We conducted a histological study and measured PAM loss and renewal after intratracheal instillation (0.2 mL) of a suspension of beryllium or aluminum hydroxides. These hydroxides were prepared from either beryllium or aluminum sulfate solutions (40 mg/mL) by adding 1 M NaOH until the pH rose to 7.3.

Observation of lung paraffin sections showed that granulomatous lesions developed within a few days. The most severe lesions were observed after 8 days. These lesions gradually disappeared and were almost absent 3 weeks after the intratracheal instillations (*28*). DNA dosage indicated that total lung cells increased by a factor of about 2 at day 8. Histology, combined with pulmonary lavage, showed that the increase in lung weight was due mainly to sequestration of migratory cells in the interstitial compartment.

We observed migration of leukocytes to the endoalveolar compartment using electron microscopy between days 1 and 3 after instillation. Different types of migrating leukocytes were involved, including monocytes. Three different migrating processes could be visualized: migration between alveolar epithelial cells, migration at sites where the alveolar epithelium had desquam-

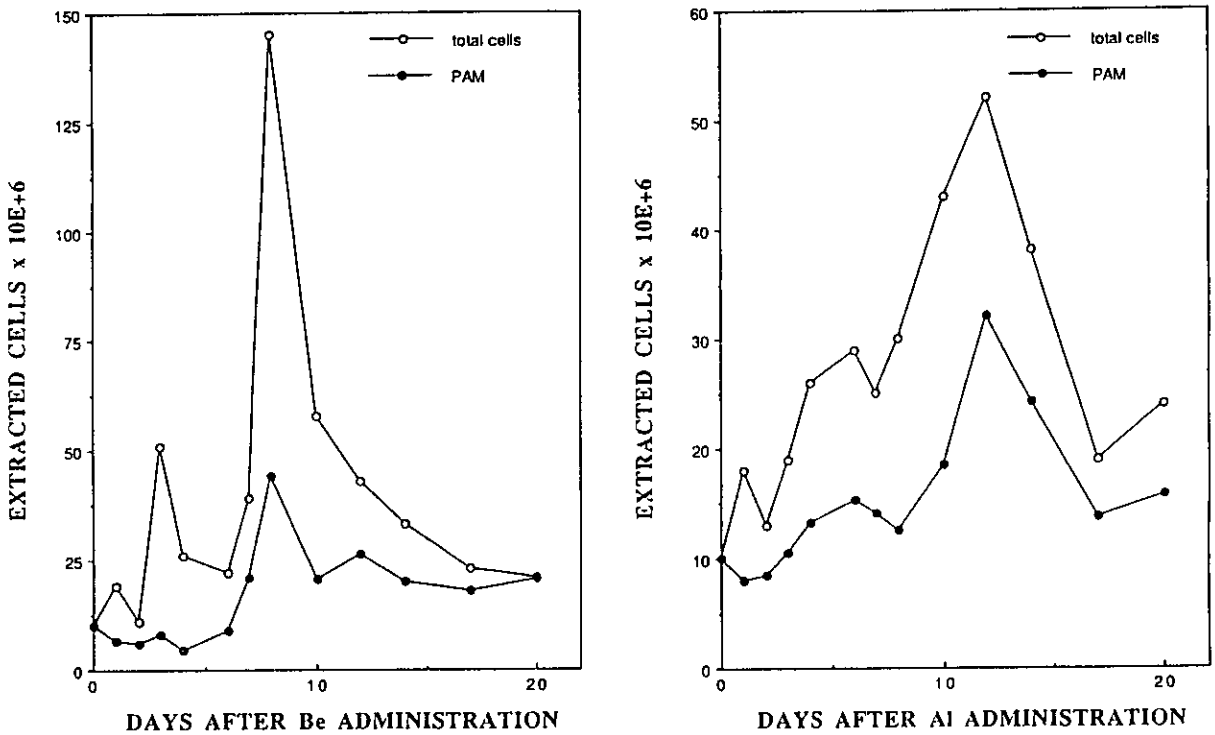

FIGURE 3. Time course of the number of endoalveolar cells extracted by pulmonary lavage after intratracheal injection of aluminum or beryllium hydroxides.

ated, and migration through the epithelial cells (emperipolesis). These migrations occurred at a similar frequency (*29*). Thus, under these experimental conditions, endoalveolar macrophages could be derived from direct migration of blood monocytes.

Figure 3 shows the time course of the number of endoalveolar cells extracted by pulmonary lavage after intratracheal instillation. The number of endoalveolar cells increased by a factor of about 15 due mainly to polymorphonuclear migration. The maximal increase of PAM peaked at about 5 times the control values. Beryllium hydroxide induced a larger migration of leukocytes to the alveoli than did aluminum hydroxide. For both chemicals, a decrease of PAM was observed during the first days after administration. This decrease was more pronounced after instillation of beryllium than after aluminum. Thereafter, the number of PAM increased sharply by a factor of 3–4 compared to controls and then gradually decreased, but remained at twice the control value 3 weeks after the intratracheal injections (*28*).

Figure 4 shows the individual clearance of radioactive hematite inhaled at subtoxic doses (50 μg) in four animals after intratracheal instillation of beryllium or aluminum hydroxides. Both compounds induced a total inhibition of hematite lung clearance. This clearance, measured between day 27 and day 67 after the inhalation, corresponded to the excretion rate of PAM via the upper respiratory tract. Two animals received an intratracheal instillation of saline and showed no alteration of their hematite clearance as compared with controls. Thus, we concluded that at the doses used, aluminum and beryllium hydroxide inhibited the migration of PAM to the upper respiratory tract within a few hours of instillation.

Some animals received ^{125}IUdR by intratracheal instillation 16 hr before the administration of either aluminum or beryllium hydroxides. A significant early decrease of ^{125}I-labeled macrophages occurred in lavaged cells after these administrations. This loss appeared earlier with beryllium- than with aluminum-treated rats. After 5 days, the clearance of PAM ^{125}I appeared to be similar that measured in controls. From these results, we estimated that aluminum and beryllium hydroxides induced the death of about half the PAM population within a few hours of administration.

Figure 5 shows the LI of PAM recovered by pulmonary lavage after incubation *in vitro* with ^{3}HT as a function of time after administration of either aluminum or beryllium hydroxides. Different LI were measured in the two animal groups: beryllium induced a significant decrease in the fraction of PAM synthesizing DNA, especially during the first week after treatment, whereas aluminum increased this fraction by a factor of 2–3 within a few hours of administration. As the subsequent evolution of PAM population was similar after beryllium or aluminum treatment, we concluded that under these experimental conditions, proliferation could not be the main factor that regulated the number of PAM.

Inhalation of Different Mineral Compounds

PAM death and excretion via the upper respiratory tract were studied after a single inhalation exposure to different mineral compounds using a Retec nebulizer. Macrophage death was studied in six groups of rats including controls: animals exposed to cadmium chloride to give initial lung burdens (internal radioative tracer added) of 15 $\pm$ 5, 200 $\pm$ 50, and 700 $\pm$ 250 μg and animals exposed to lead acetate to give initial lung burdens of 200 $\pm$ 50 and 700 $\pm$ 250 μg. ^{125}IUdR labeling of PAM was

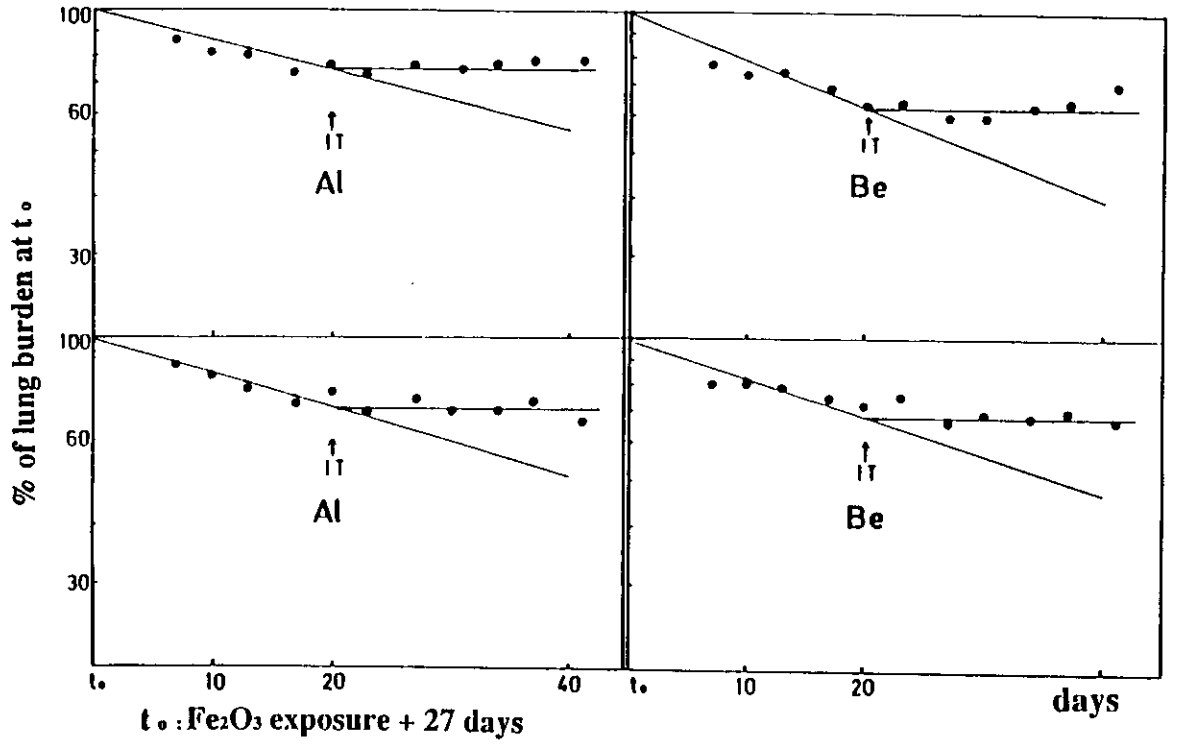

FIGURE 4. Effects of intratracheal injection of aluminum or beryllium hydroxides on individual clearance of inhaled radioactive hematite.

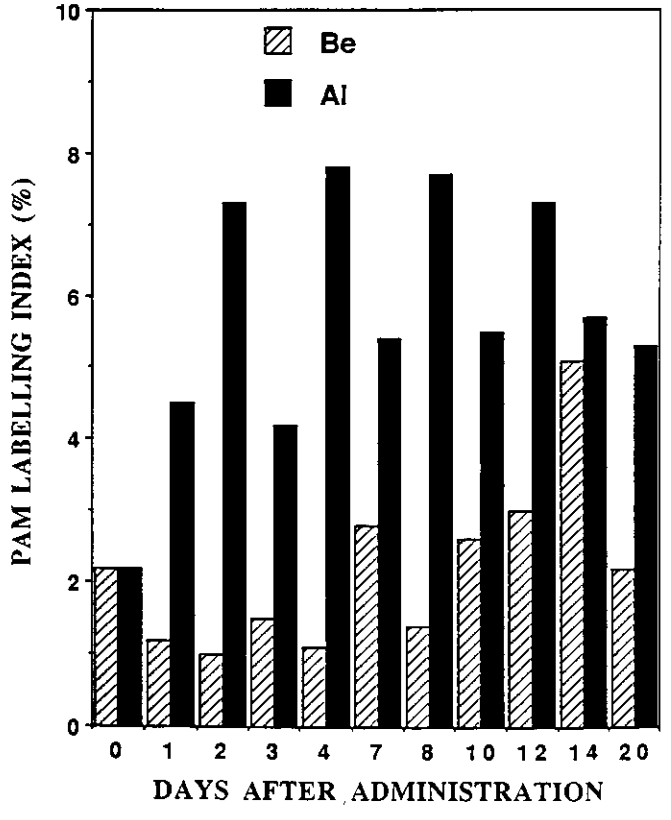

FIGURE 5. Percentage of PAM synthesizing DNA as a function of time after intratracheal injection of aluminum or beryllium hydroxides.

performed 24 hr before exposure to these different materials. Only the animal group with the initial lung burden of 700 μg of cadmium chloride showed a significant decrease in the amount of ^{125}I recovered after pulmonary lavage. In these lethally exposed animals, a decrease of ^{125}I labeling was observed within a few hours after inhalation, and this was more pronounced (about 80%) than the decrease observed after intratracheal instillation of aluminum or beryllium hydroxides. The lack of significant PAM death after inhalation of cadmium chloride at the initial lung burden of 200 μg is surprising; it indicates that cytotoxicity in macrophages measured with IUdR is a poor index of lung toxicity of cadmium, and the same is true for lead.

Cell migration via the upper respiratory tract was studied by measuring ^{59}Fe lung clearance in three groups of rats that inhaled hematite, carbon, or silica. All these animals had previously inhaled 50 μg of radioactive hematite. The results are shown in Figure 6. Increasing the hematite lung burden to values as large as 15 mg/g of lungs did not modify the clearance of ^{59}Fe (mean half-life about 35 days, measured after the initial upper respiratory tract clearance). Silica inhalation did not modify ^{59}Fe clearance up to a lung burden of about 1 mg/g of lungs. Silica burdens between 1 and 3 mg induced a gradual increase in clearance, and, for lung burdens larger than 3 mg, hematite clearance remained at a nearly constant value, with a half-life of about 210 days. A similar phenomenon was observed after inhalation of coal dust. The clearance was altered for lung burdens up to 4 mg/g of lungs and remained at a nearly constant value (half life about 300 days) for lung burdens up to 10 mg.

These results show that clearance parameters of radioactive particles can be used to characterize the effect of inhaled particles

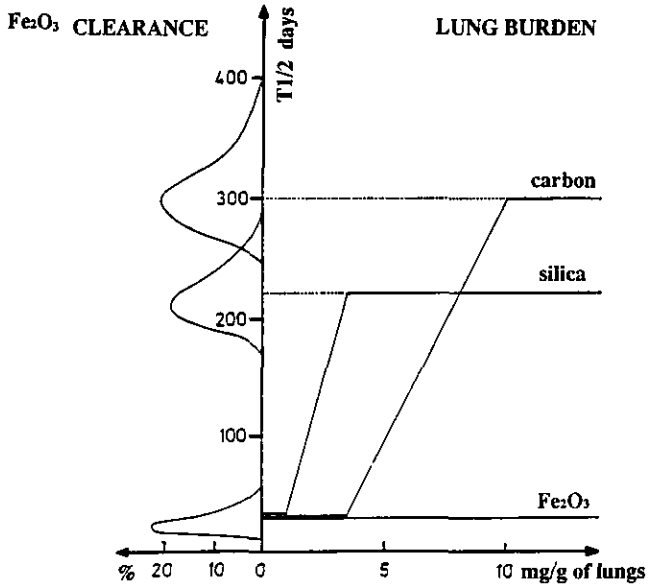

FIGURE 6. Effects of inhalation of increasing amounts of hematite, silica, or carbon on the clearance of radioactive hematite.

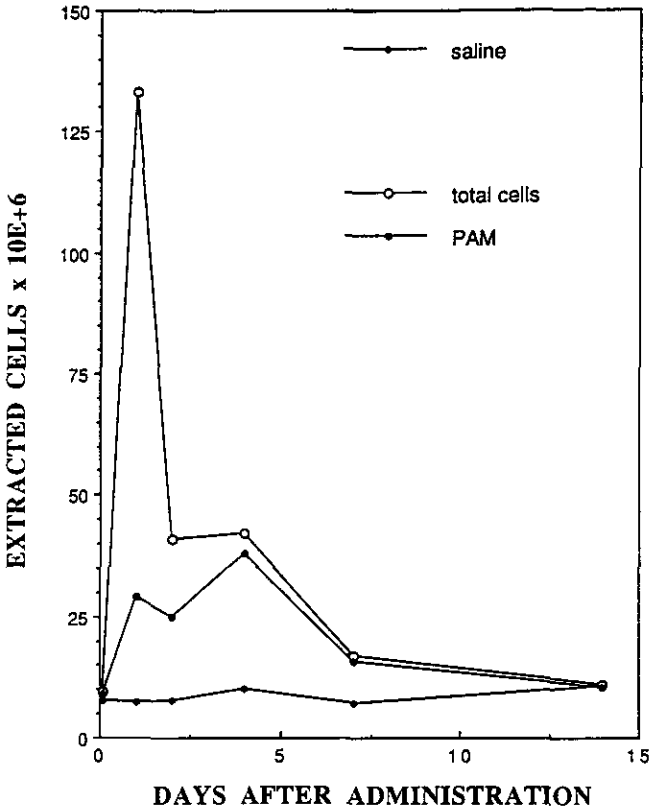

FIGURE 7. Time course of the number of endoalveolar cells extracted by pulmonary lavage after intratracheal injection of bacteria.

on PAM kinetics. Toxic particles inhibit PAM migration to the upper respiratory tract as a function of inhaled doses. The dose–effect relationship for clearance inhibition depends on the type of the particle studied and is likely to reflect cell toxicity. The alterations of half-life clearance to values at about 200 to 300 days, similar to the half-life measured in controls from 3 to 4 months after inhalation, might correspond to the rate of particle dissolution.

Intratracheal Injection of Bacteria

We studied the effect on PAM renewal of the intratracheal instillation of 30×10^6 *Staphylococcus aureus* in 0.15 mL saline (*30*). The strain used (P 50-82 from Institut Pasteur) is coagulase-positive, sensitive to penicillin and streptomycin, and does not produce enterotoxine. PAM synthesizing DNA were first labeled by intratracheal instillation of ^{125}IUdR. Either bacteria or saline were administered to rats 3 days after this labeling. Sequential killing was performed at 2.5 hr or 1, 2, 3, 4, 7, and 14 days. Lungs were lavaged after *in vitro* incubation with ^{3}HT to label PAM in S-phase. Clearance of viable bacteria from the lungs was measured. At 3 hr after administration, from 500 to 70,000 viable bacteria were extracted from lavaged lungs. After 24 hr, 4200 (SD = 2300, $n = 3$) bacteria could still be extracted, but no viable bacteria were extracted lungs lavaged from days 2 to 14 after treatment.

Figure 7 shows the evolution of the endoalveolar cell population extracted by pulmonary lavage as a function of time after treatment. In contrast to results obtained after intratracheal injection of either aluminum or beryllium hydroxides, bacterial administration induced a transient but totally reversible change in the endoalveolar cell population. Endoalveolar cells extracted by pulmonary lavage on day 14 were qualitatively and quantitatively similar to controls and to rats sham-treated with saline. Administration of bacteria induced a large migration of cells to the endoalveolar compartment, which was due mostly to a transient migration of polymorphonuclear cells starting within a few hours after treatment. After 1 week, polymorphonuclear cells were less numerous than PAM and, after 2 weeks, they had nearly disappeared. A 3-fold increase in the number of PAM was induced within 1 day after treatment. This significant enhancement of the PAM population was still observed after 1 week. During that time, a modification of usual PAM morphology was observed, with most PAM being much smaller than in controls. Measurement of cell loss after ^{125}IUdR labeling did not show any significant difference between bacteria-instilled and saline-instilled groups. Thus, administration of bacteria did not result in a measurable excess of PAM death.

Intratracheal injection of bacteria altered the fraction of PAM synthesizing DNA, whereas after saline administration, this fraction remained constant, at a value similar to that measured in controls (Fig. 8). A significant increase of ^{3}HT-labeled cells was observed within 2.5 hr of bacteral instillation. The labeling index peaked at 1 day after administration and decreased thereafter. This increased number of PAM synthesizing DNA could not explain the increase in PAM population from about 10×10^6 to 30×10^6 within 24 hr. Assuming a 12-hr S-phase, the maximal local PAM production should be about 3×10^6 on day 1. These results confirm those obtained after administration of beryllium hydroxide and show that the increased PAM population was due mainly to monocyte migration to the endoalveolar compartment. After bacteria instillation, this new migrating

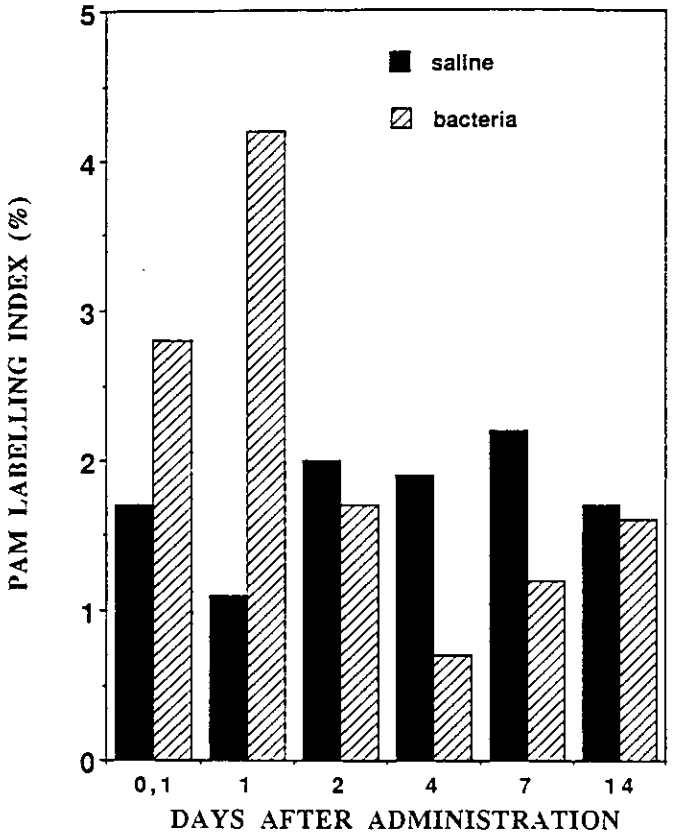

FIGURE 8. Percentage of PAM synthesizing DNA as a function of time after intratracheal injection of bacteria.

PAM population was quite different from the original PAM population, not only morphologically and possibly functionally, but also in life span. Assuming that the original PAM population remained at a constant value (about 10×10^6), the new migrating PAM population decreased from 30×10^6 to 10×10^6 between days 4 and 7 after bacterial instillation. The calculated half-life of this new migrating population was about 1.8 days, which is 5 times shorter than that measured for normal PAM.

Conclusions

This study of PAM renewal under normal and pathological conditions provides new experimental procedures that could be useful for *in vivo* measurement of induced PAM toxicity after inhalation of different types of particles. In addition to ^{3}HT labeling, two kinetic assays can be used: measurement of cell migration to the upper respiratory tract and measurement of PAM loss, mainly corresponding to PAM death in the endoalveolar compartment. As they were performed, these assays provided semiquantitative information on the intra-alveolar toxicity of inhaled or instilled particles as a function of their burden deposited in the lungs. Further studies are needed to develop more sensitive biological indicators.

Under normal conditions, the observed results confirm the hypothesis we formulated previously (*15*), i.e., that most PAM are derived from the migration of precursors that divide only once in the endoalveolar compartment. This division can be considered as a maturation process. These precursors are not blood monocytes but proliferative cells that could be derived from monocytes. Under normal conditions, interstitial macrophages are unlikely to correspond to the PAM precursors, and intracapillary monocytes are the best candidates.

During pathological conditions, all parameters of PAM homeostasis can be altered. In all of the experimental conditions studied, local proliferation of PAM always appeared to be a minor contribution to renewal number of when an increased PAM was observed, and significant PAM death was always associated with acute lung pathology.

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