DISTRIBUTION OF LATEX PARTICLES IN LUNG AND LYMPH NODE TISSUES OF RATS AND DOGS

PRINCIPAL INVESTIGATORS
H.-L. Mueller
B. A. Muggenburg
N. A. Gillett
R. A. Guilmette

Abstract — The distribution of fluorescent polystyrene microspheres in different lung compartments, with differing particle numbers within individual lung and lymph node cells was examined in methacrylate-embedded tissue slices. Rat tissues were analyzed at 1, 7, and 13 days after particle instillation, dog tissues at 7 and 13 days. A much higher fraction of particles was seen in the lung interstitium and in lung-associated lymph nodes in dogs than in rats. Particle concentrations in TBLN cells were generally very low in both species, but were high in free alveolar cells after high particle numbers were instilled, and increased from 1 to 13 days, suggesting that alveolar cells with few particles were cleared faster than those with high ingested particle numbers.

Fluorescent-labeled latex particles are useful for investigating particle distributions in lung and lymph node tissues using epifluorescent microscopy. This study was done to investigate potential species differences in particle distributions in the different lung compartments, especially with regard to translocation to the lung interstitium, for species with different particle clearance and lymph node translocation rates. Rats and dogs were chosen for this study because dogs clear particles very slowly from the lung, whereas rats clear particles much faster, but translocate smaller fractions of inhaled particles to their tracheobronchial lymph nodes.[1] Changes in particle distributions in free lung cells over time can also be studied with fluorescent particles, and could deliver information about how particle numbers inside cells change during lung clearance, and if they could influence the abilities of macrophages to leave the lung.

METHODS

Twelve male Fischer 344/N rats, 12-14 wk old, and 2 male Beagle dogs, 7-9 yr old, were used in this study. Monodisperse, green or blue fluorescent, polystyrene microspheres with a geometric diameter of 1.7 μm were used throughout the study. Six rats each were instilled with either 0.8×10^9 green fluorescent particles or 1.1×10^7 green fluorescent particles, suspended in 0.5 mL physiological saline while under halothane anesthesia. Groups of two rats from each particle dose were sacrificed at 1, 7, and 13 days. The lungs and tracheobronchial lymph nodes (TBLN) were removed and fixed with 10% ice-cold buffered formalin.

Individual lung lobes of one dog were instilled with 0.8×10^7 blue fluorescent particles at 48 days, 1.6×10^7 blue fluorescent particles at 42 days, and 1.3×10^9 green fluorescent particles at 13 days before sacrifice. Different lung lobes of a second dog were instilled with 1.3×10^9 green fluorescent particles, either at 13 days or at 7 days before sacrifice. Instillations were done with a fiberoptic bronchoscope, while the dogs were under halothane anesthesia, as previously described.[2] Some of the lung lobes studied were lavaged to obtain macrophages for mobility studies (this report, pp. 137-140). Lung lobes and left upper and middle TBLNs were fixed with 10% ice-cold buffered formalin.

Rat and dog tissues were embedded in glycol methyacrylate (Sorvall embedding medium) at 4°C. Sections 1.5-μm thick were made for epifluorescent light microscopic studies. Whole lung slices were analyzed for particles following the instillation of low particle numbers. Ten randomly chosen microscopic fields at a 250-fold magnification were analyzed in slices of lungs instilled with high particle numbers. Particles in free alveolar cells, interstitial locations, and in airways were counted in the lung slices. All particles in whole TBLN slices were counted and particle numbers in individual cells were also recorded. Frequency distributions of particles in cells and particle concentrations in cells from the different locations were calculated.

RESULTS AND DISCUSSION

Table 1 shows results for rats with high (Table 1a) and low (Table 1b) instilled particle numbers. Table 2 shows the results for dogs. With high numbers of instilled particles there was a decrease in free lung cells containing low particle numbers and an increase in cells containing high particle numbers between 1 and 13 days. These changes correlate with the observation that it was mainly lavaged lung cells with low ingested particle numbers that were able to migrate *in vitro* (this report, pp. 137-140). This suggests a possible participation of active macrophage

Table 1a

Particle Distributions in Free Alveolar, Interstitial, and Tracheobronchial Lymph Node (TBLN) Cells in the Rat Lung After High Dose Instillation[a]

(means ± SE, n = 2)

	Time After Instillation, Days	n =	% of Particle-Containing Cells with n Particles				
			1-2	3-4	5-9	10-15	>15
Free alveolar cells	1		39.9 ± 2.8	21.3 ± 0.7	20.6 ± 2.6	12.5 ± 4.5	5.7 ± 1.5
	7		20.5 ± 0.5	17.6 ± 2.3	28.9 ± 1.5	19.4 ± 1.7	13.7 ± 1.7
	13		17.5 ± 1.0	10.2 ± 1.6	31.0 ± 6.2	23.9 ± 3.0	17.4 ± 3.9
Interstitial cells	1		88.9 ± 11.1	5.6 ± 5.6	5.6 ± 5.6	0.0	0.0
	7		94.0 ± 6.0	6.0 ± 6.0	0.0	0.0	0.0
	13		85.6 ± 4.4	10.4 ± 1.4	3.9 ± 2.8	0.1 ± 0.1	0.0
TBLN cells	1		88.7 ± 4.3	5.2 ± 5.2	6.1 ± 1.0	0.0	0.0
	7		--	--	--	--	--
	13		96.6 ± 3.5	3.0 ± 3.0	0.5 ± 0.5	0.0	0.0

	Time After Instillation, Days	Average Particle No./ Particle-Containing Cell
Free alveolar cells	1	5.6 ± 0.8
	7	8.5 ± 0.3
	13	9.2 ± 0.9
Interstitial cells	1	1.6 ± 0.6
	7	1.5 ± 0.7
	13	1.7 ± 0.2
TBLN cells	1	1.4 ± 0.1
	7	--
	13	1.2 ± 0.1

[a]Determined in 1.5 μm tissue slices at 1, 7, or 13 days after intratracheal instillation of 0.8×10^9 fluorescent-labeled polystyrene microspheres (1.7 μm) in 0.5 mL saline into whole lungs.

Table 1b

Particle Distributions in Free Alveolar, Interstitial, and TBLN Cells in the Rat Lung After Low Dose Instillation[a] (means ± SE, n = 2)

	Time After Instillation, Days	n =	% of Particle-Containing Cells with n Particles				
			1	2	3	4	>4
Free alveolar cells	1		66.3 ± 3.6	21.9 ± 0.1	6.7 ± 1.4	2.8 ± 0.4	2.3 ± 1.8
	7		63.1 ± 4.5	19.4 ± 0.8	7.4 ± 0.1	2.7 ± 0.0	2.4 ± 0.3
	13		70.0 ± 2.9	22.0 ± 0.7	4.5 ± 1.2	1.6 ± 0.6	1.9 ± 0.5
Interstitial cells	1		100.0 ± 0.0	0.0	0.0	0.0	0.0
	7		75.0 ± 25.0	25.0 ± 25.0	0.0	0.0	0.0
	13		95.7 ± 4.4	4.3 ± 4.3	0.0	0.0	0.0
TBLN cells	1		100.0 ± 0.0	0.0	0.0	0.0	0.0
	7		91.0 ± 1.0	7.7 ± 2.3	1.3 ± 1.3	0.0	0.0
	13		100.0 ± 0.0	0.0	0.0	0.0	0.0

	Time After Instillation, Days	Average Particle No./ Particle-Containing Cell
Free alveolar cells	1	1.57 ± 0.20
	7	1.51 ± 0.04
	13	1.45 ± 0.07
Interstitial cells	1	1.00 ± 0.00
	7	1.14 ± 0.14
	13	1.05 ± 0.05
TBLN cells	1	1.00 ± 0.00
	7	1.11 ± 0.01
	13	1.00 ± 0.00

[a]Determined in 1.5 μm tissue slices at 1, 7, or 13 days after intratracheal instillation of 1.1×10^7 fluorescent-labeled polystyrene microspheres (1.7 μm) in 0.5 mL saline into whole lungs.

movement in lung clearance, but the reason for the observed changes in particle distributions could have also been due to particle redistributions after macrophages died and released their loads, followed by rephagocytosis by other macrophages.

Interstitially deposited particles in the rat lung were almost exclusively found in cellular regions in the peribronchial interstitial tissue, often adjacent to larger blood vessels. The regions are thought to be lymphocyte aggregates, and some of them resemble the bronchus-associated lymphoid tissue (BALT) described by Bienenstock et al.[3] These structures were rarely seen in dog lung tissue, and interstitial particles were mostly observed around blood vessels (up to 50% of all interstitial particles), and in the peribronchial interstitial tissue in regions with low numbers of interstitial cells.

TBLN cells contained low particle numbers at all times and with both particle doses. Particle concentrations in TBLN cells were somewhat higher in the dog than in the rat, and a slight nonsignificant decrease was observed in both species from 1 or 7 to 13 days (Tables 1a and 2a).

With high instilled particle numbers, great numbers of particles were present in the lung interstitium of dogs at 13 days after instillation, whereas only a few particles were seen in the rat lung interstitium at 1, 7, and 13 days after the instillation of high particle numbers. Quantitation was not attempted with tissue samples from the animals with high particle numbers.

Table 2a

Particle Distributions in Free Alveolar Cells and TBLN Cells in the Dog After High Dose Instillation[a] (mean values ± SE from 2 animals)

	Time After Instillation, Days	n =	% of Particle-Containing Cells with n Particles 1-2	3-4	5-9	10-15	>15
Free alveolar cells	7[b]		26.8	17.5	27.8	13.6	14.1
	13		18.2 ± 1.8	14.7 ± 4.3	30.9 ± 4.1	18.7 ± 4.6	17.5 ± 5.6
TBLN cells	7[b]		82.6	8.1	5.1	3.0	1.3
	13		83.2 ± 6.7	10.6 ± 3.3	4.7 ± 1.9	1.0 ± 1.0	0.5 ± 0.5

	Time After Instillation, Days	Average Particle No./ Particle-Containing Cell
Free alveolar cells	7[b]	7.95
	13	9.06 ± 1.52
TBLN cells	7[b]	2.24
	13	1.90 ± 0.41

[a]Determined in 1.5 μm tissue slices at 7 or 13 days after intratracheal instillation of 1.3×10^9 fluorescent-labeled polysytrene microspheres (1.7 μm) into individual lung lobes. Lung lobe lavaged immediately before embedding at 7 days; no lavage at 13 days.

[b]Single values.

Table 2b

Particle Distributions in Free Lung Cells and TBLN Cells in the Dog After Low Dose Instillation[a]

	n =	% of Particle-Containing Cells with n Particles 1	2	3	4	>4
Free lung cells[b]		84.0	14.2	1.8	0.0	0.0
Free lung cells[c]		98.4	1.6	0.0	0.0	0.0
TBLN cells		88.8	4.6	4.5	0.0	2.1

	Average Particle No./ Particle-Containing Cell
Free lung cells[b]	1.18
Free lung cells[c]	1.02
TBLN cells	1.22

[a]Determined in 1.5 μm tissue slices at 42 or 48 days after intratracheal instillation of fluorescent-labeled polystyrene microspheres (1.7 μm) into individual lung lobes of the same dog.

[b]1.6×10^7 particles in 1 mL saline instilled 42 days before sacrifice.

[c]0.8×10^7 particles in 1 mL saline instilled 48 days before sacrifice. Each lung lobe was lavaged at 1 day after the instillation.

With low particle numbers, scanning of whole lung slices was done and quantitative distribution patterns were obtained (Table 3). The fraction of deposited particles in the rat lung interstitium was low from 1 to 13 days, but showed a slow increase during this time interval. We have no samples from dogs for the same time points, but the interstitial particle fraction in the dog lung was about ten-fold higher at 42 days after particle instillation than at 13 days in the rat, and extraordinarily high at 48 days after instilling a two-fold lower particle number. As lung clearance of insoluble particles is slower in the dog than in the rat,[1] these high fractions of interstitial particles could not have been the result of a ratio change due to a fast removal of particles from the alveoli via the mucociliary escalator.

Table 3
Percentage of All Particles in Analyzed 1.5 μm Lung Tissue Slices
from Rats and Dogs Found in the Interstitium and in Airways[a]
(means ± SE; n = 2)

Time After Instillation	Species	Number of Instilled Particles x 10^7	Fraction of Particles (%) in Alveoli and Respiratory Bronchioles	Respiratory Interstitium[b]	Ciliated Airway Lumen
1 day	Rat	1.08	99.39 ± 0.15	0.32 ± 0.11	0.29 ± 0.02
7 days	Rat	1.08	98.92 ± 0.62	0.59 ± 0.23	0.48 ± 0.39
13 days	Rat	1.08	97.76 ± 0.50	1.76 ± 0.81	0.47 ± 0.32
42 days	Dog	1.60	80.02[c]	18.81[c]	1.17[c]
48 days	Dog	0.78	50.00[c,d]	50.00[c,d]	0.00[c,d]

[a]After intratracheal instillation of fluorescent-labeled polystyrene microspheres (1.7 μm). Rat lungs were not lavaged; dog lungs were lavaged at 1 or 7 days after particle instillation.

[b]Including airway walls, blood vessel walls, and alveolar wall interstitium.

[c]Single values.

[d]Very low particle numbers were found in the whole slice.

Our data suggest that one reason for enhanced particle translocation to TBLNs and reduced clearance from the lungs of dogs may be the presence of a large fraction of inhaled particles in the dog lung interstitium, from which clearance is extremely slow compared to clearance of particles in the alveoli.[4] Furthermore, if particle translocation to the lymph nodes is associated with migration of alveolar macrophages, as suggested by Harmsen et al.,[5] macrophages with very low numbers of ingested particles should have the highest ability to cross the alveolar epithelium and migrate to the TBLNs.

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