Is Lung Sequestration of Indium-111-Labeled Granulocytes Organ Specific?

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Transient sequestration of polymorphonuclear leukocytes (PMN) in the normal lungs of animals occurred immediately following intravenous injection of ^{111}In-labeled PMN. We investigated the organ specificity of this process. Equal amounts of homologous PMN, derived from the intravascular space and labeled with [^{111}In]oxine, were infused either intravenously (i.v.) or intraarterially (i.a.) into pairs of rats. Changes in radioactivity emitted from three regions—representing lung, liver and spleen, and lower body—were determined from images during the following hour. A nonspecific character was demonstrated by the transient sequestration of activity in the lower body following i.a. infusions. However, the rate of initial clearance of activity (first 30 min) from the lungs of i.v.-infused rats was relatively slower than from the lower body of i.a.-infused rats. This suggests the presence of a lung-specific as well, which may be important for localization of PMN-related events to the lung.

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Polymorphonuclear leukocytes (PMN) labeled with indium-111 (In-PMN) are utilized clinically to identify inflammatory processes, and experimentally to examine in vivo PMN kinetics and in vitro PMN migration (*1–15*). Following injection of In-PMN into a peripheral vein, transient sequestration of activity is apparent in the lung region. Although lung retention of activity is increased by lung injury (*11–14*), transient sequestration is apparent in normal lungs as well. The significance of this lung sequestration in normal lungs is unknown.

To the extent that labeled cells may have been stimulated by the separation and labeling process, they may reflect the migratory patterns of partially-activated or otherwise perturbed PMN. Although activated PMN may circulate in some lung injury processes in which PMN may play a pathogenic role (*16–19*), symptoms of lung injury are not usually induced by infusion of In-PMN. Although lung sequestration has been noted with all labeled cell preparations, the magnitude and/or duration of this process appears to vary from individual to individual, between various cell preparations, and with the methods of cell isolation, separation, and labeling (*8,10–12,20*). However, some degree of lung sequestration has been noted following infusion of virtually all labeled PMN preparations, perhaps a result of in vitro handling and labeling.

Whether transient sequestration of In-PMN occurs in normal lungs simply because they represent the first microvascular bed which these cells attempt to cross, or whether this sequestration is specific to the lung, has not been previously demonstrated. Likewise, the organ specificity of lung sequestration of mobilized and potentially activated neutrophils is not well-established (*21*). On one hand, i.v. infusion of leukocytes activated in vitro, or of substances which can activate leukocytes, have been reported by some to lead to lung sequestration of PMN (*22–24*). On the other hand, when others examined extrapulmonary microvascular beds after i.v. infusion of cobra venom factor (which activates PMN), sequestration was not lung specific (*25*).

The purpose of this study was to determine the relative, organ specificity of transient sequestration of activity in the lung following intravenous infusion of In-PMN. To address this question, we examined the relative rates of change in activity of pulmonary and nonpulmonary regions of rats, as occur following either arterial (aorta) or venous (femoral) infusion of homologous PMN-labeled with [^{111}In]oxine.

METHODS

Animals

Fifteen, respiratory-disease free, Sprague-Dawley rats (Charles River Laboratories, Worcester, MA) weighing 325–350 g, were used in this study.

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Cell Harvest

Homologous PMN were obtained from five rats by intravascular exchange with heparinized 6% hetastarch in 0.9% NaCl (American Critical Care, McGraw Park, IL), using methods reported previously (*10–11*). Briefly, repeated exchanges of 2 ml of hetastarch containing heparin for equal volumes of blood were performed through the femoral vein until the animal succumbed.

Cell Separation

The cells were separated using a double-density, ficoll-hypaque (FH) gradient, using methods previously reported (*3, 4,10,11*). Briefly, red cells were sedimented in hetastarch to gravity. PMN were isolated at the interface of FH layers of specific gravities 1.077 and 1.135 after 20-min centrifugation at 450 *g*, and washed once in saline. The plasma-hetastarch supernatant (PHS) was saved.

Cell-Labeling

The cells were labeled with [^{111}In]oxine (Amersham, Arlington Heights, IL), using methods previously described (*3,4, 10,11*). Centrifugation steps were performed onto a small "button" of FH (s.g. 1.135) to minimize cell trauma (*3*), with only gentle rocking generally necessary for resuspension. The cells were washed once with saline. The cells were then washed and resuspended in the plasma-hetastarch supernatant (PHS) obtained during cell separation for infusion (*10,11*), and passed through a blood component filter for infusion.

Labeled cell suspensions were examined microscopically prior to infusion, and aggregates were not seen. Cell viability was found to be >98% by trypan-blue dye exclusion in all samples. Cell associated activity was 96% (± 2), with PMN representing 98% (± 0.8) of nucleated cells. With these methods, which avoid hypotonic lysis, RBC-associated activity is negligible (*10,11*).

Indium-111 PMN Infusion

To minimize the effects of variability in performance of various cell suspensions (*8,11,12*), the cell suspensions were divided equally for injection into a pair of animals, with one rat receiving cells i.v. and one receiving cells intraarterially (IA). Because the animals were reportedly inbred, they should share very similar genetic profiles, and transfusion reactions were not evident clinically. Ten animals were injected with In-PMN, individually receiving a mean of 8.9 (± 2.4) × 10^6 PMN, in 1.2 (± 0.1) cc PBS. Intravenous (i.v.) injections were made into the femoral vein via cutdown. Intraarterial (i.a.) injections were made into the lower abdominal aorta via midline abdominal incision. Indium-PMN were infused over ~ 30–45 sec. All procedures, including subsequent scanning, were performed under ketamine anesthesia (Parke-Davis, Arlington Heights, IL).

Imaging

Animals were scanned in the posterior view, and data acquisition begun within ~ 1 min of injection. Photographic records of emitted activity as detected by a gamma camera (Picker, Northford, CT) were obtained intermittently following each infusion. Activity was also continuously recorded at maximally 4-min intervals for ~ 1 hr by a MUGA computer (Picker, Northford, CT) on-line to the gamma camera. Regions of interest were subsequently identified (Fig. 1) to represent activity from the upper portion of the lungs (Lu), the lower end of the body (designated LE), and liver-spleen (LS) region.

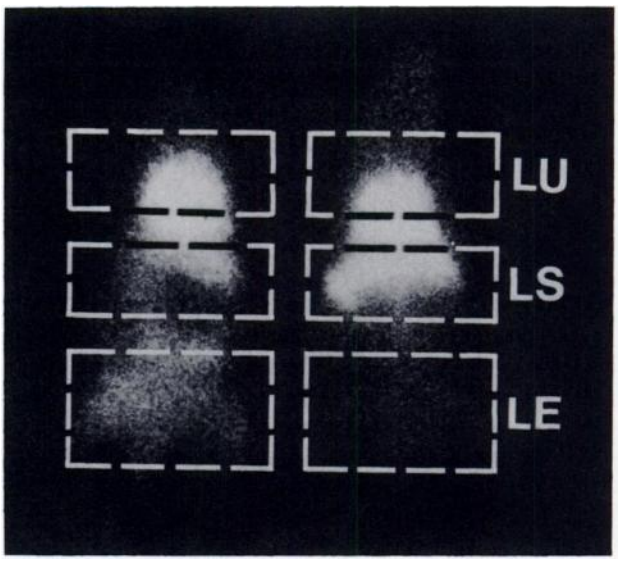

FIGURE 1
Regional activity. Pictured is a polaroid exposure of emissions obtained ~ 5 min following infusion of indium-labeled PMN in a pair of rats. The image on the left represents a rat infused intraarterial, and on the right a rat infused intravenously. The regions of interest include the lungs (LU), the liver and spleen (LS), and the lower end of the body (LE).

Activity was normalized to the maximal regional value in each animal, and relative changes in regional activity identified. This approach compensated for unavoidable differences between animals regarding (a) differences in activity/cell of various cell preparations, and (b) the distance to, or proportion of, the organs identified by each region. While normalization of regional activity to that of the injectate (*13*) would correct for the first, it would not correct for the second source of error. The approach described here also minimized problems in estimating actual whole lung activity, which may be affected by scatter from the adjacent liver and spleen, or reproducibly identifying identical regions of activity in different rats.

Data Analysis

The changes in regional activity sequentially reflect relative rates of accumulation and/or clearance of labeled PMN in the various regions identified in the two groups. Values reported represent means (± standard errors). Data are expressed logarithmically when activity clearance was examined (*26*). These data were analyzed for variance and covariance of repeated measures using a statistical software package (BMDP 2V; UCLA, CA). Differences were accepted as statistically significant when the probability of each group being part of the same distribution was < 5% ($p < 0.05$).

RESULTS

Sequential images of a pair of animals obtained at several intervals following injection of In-PMN are shown in Figure 2, with images A-D photographed

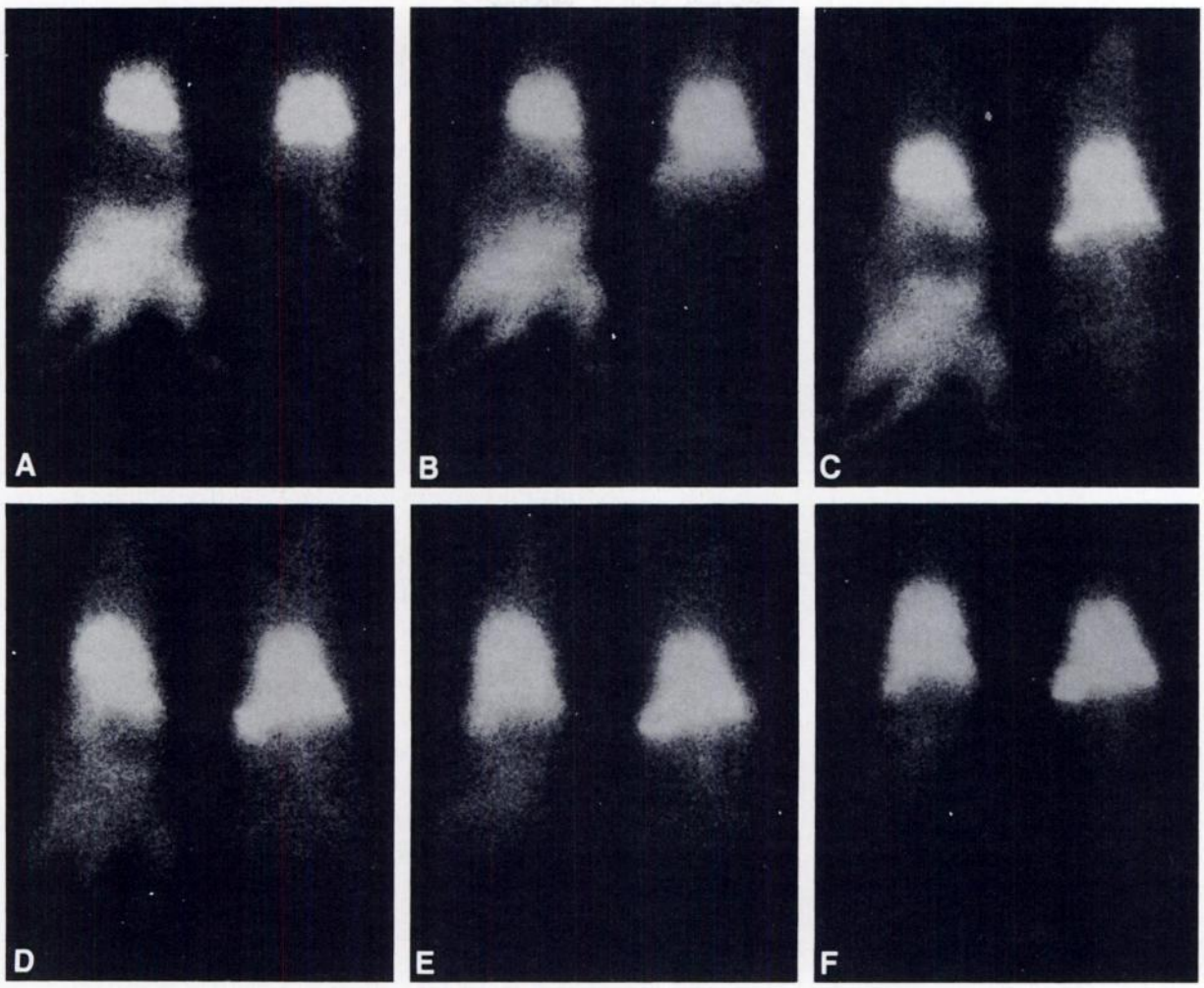

FIGURE 2
Photographic images. Sequential images obtained following infusion of labeled cells are shown here. As in Figure 1, cells were infused intraarterially on the left, and intravenously on the right. The first four images (A-D) were obtained during the initial 15 min, with additional images representing ~ 30 (E) and 60 (F) min following infusion.

during the first 15 min. In animals receiving labeled cells i.a., transient sequestration of activity in the lower body (primary microvascular bed) is evident, but activity in this region rapidly declines. Concurrently, activity in the lungs, representing the second microvascular bed which these cells must cross, appears quickly, peaking early and declining less rapidly. In contrast are animals receiving labeled cells i.v. Activity in the primary bed, the lungs, is intense immediately following injection, but remains relatively prominent through a less dramatic decline. Concurrently, activity in the second microvascular bed of these animals, the lower body, accumulates relatively slowly and less prominently than was apparent in the lungs following i.a. injection. Meanwhile, in both groups of animals, activity in the region of the liver and spleen gradually accumulates during this early phase, although activity appears to accumulate more rapidly in this region following i.v. than i.a. infusion of labeled cells. After 30 min (Figs. 2D-E), the images differ little between the two animals, with regional activity changing relatively little in both groups.

Sequential changes in computer-quantitated, relative, regional activities are shown in Figure 3. It is important to remember that the values represent means of relative (not absolute) activity in that region, as a percent of maximum regional activity imaged during any 4-min interval in each animal. The rates of change in regional activity are the focus of this study, not the absolute regional activity, as described in the methods above and discussed again below.

Transient sequestration of activity in the primary bed of both groups, including the lungs of rats receiving cells i.v. and the lower extremity (lower body) in rats receiving cells i.a., can be appreciated by two characteristics of the curves. First, there is a relatively rapid decrease in regional activity noted in both primary vascular beds initially (Figure 3A). Second, there is simultaneously early increase in activity found in the

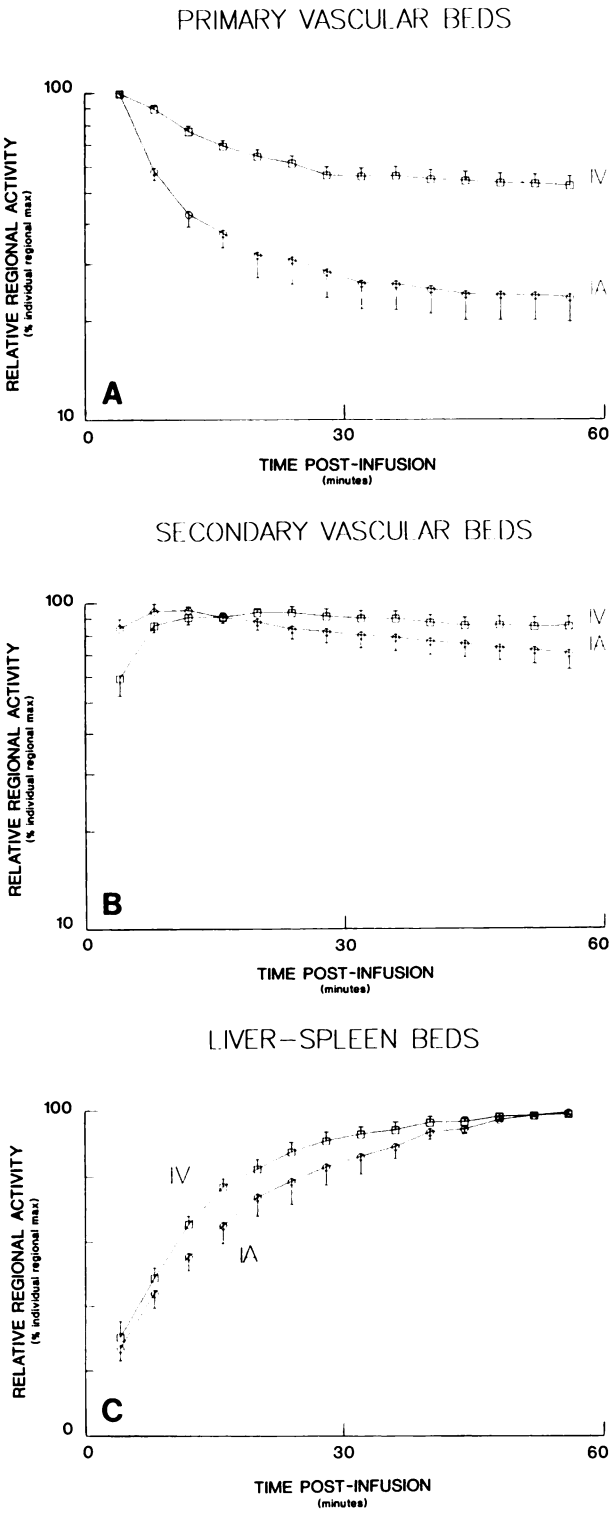

FIGURE 3
Computer-recorded, relative-regional activity. Sequential activity is shown. Values are normalized to the maximal regional activity measured in one 4-min, time interval during the hour of scanning of that animal. The data shown represent means and standard errors from groups of five animals. Regions compared included the following. A: Primary bed activity. Changes in activity in the initial, or primary bed that the labeled cells traverse, are displayed here. The primary bed of the rats infused i.v. is represented by the lung region, while that of rats infused i.a. is represented by the lower body. Activity is plotted on a semilogarithmic scale against time, to reflect clearance rates. Changes in activity over time of these primary beds differed significantly ($p < 0.001$) between the two groups during the initial 30 min but not subsequently. B: Secondary bed activity. Changes in activity in the secondary beds, which the labeled cells would traverse after clearing the primary bed, are displayed here as in Figure 3a. In rats infused i.v., the lower body reflects the secondary bed, and in those infused i.a., the lungs reflect the secondary bed. Changes in activity of these secondary beds again significantly differed ($p < 0.01$) between the two groups during the initial 30 min. The differences were no longer statistically significant after 30 min. C: Liver-spleen activity. Changes in activity in the liver-spleen regions, presumed sites of PMN margination and clearance, are displayed here. Although the initial accumulation of activity in this region tended to occur more slowly following i.a. infusion, these differences did not reach statistical significance ($p > 0.10$).

secondary beds of both i.v. (lower body) and i.a. (lung) infused animals (Fig. 3B).

However, during the initial 30 min, differences between the two groups can be identified regarding the rate of change in activity in these primary and secondary beds. In the primary beds, the rate of decline in activity in the lung region of i.v.-rats was significantly slower ($p < 0.001$) than from the lower extremities of i.a.-rats (Fig. 3A). This difference was noted with every cell preparation. Concurrently in the secondary vascular beds (Fig. 3B), activity peaked later and declined more gradually in the lower body of i.v.-animals than from the lungs of i.a.-animals ($p < 0.01$).

In contrast, after the initial 30 min, activity decreased more gradually in the primary and secondary beds of both groups, with some individual variability observed in the secondary beds. The changes in mean activity values of the beds after 30 min were not significantly different ($p > 0.30$).

The liver-spleen regions did not demonstrate statistically significant differences throughout the hour of scanning. Activity was noted to accumulate at a declining rate in this region in both i.a. and i.v. animals (Fig. 3C). Although accumulation of activity in this region did appear to occur more slowly in animals receiving cells intraarterially during the initial 30 min, this was not statistically significant ($p > 0.10$).

DISCUSSION

These results demonstrate both a nonspecific and lung-specific character to In-PMN sequestration. Transient sequestration of activity was evident in the lower body when these cells were injected into the arterial circulation, demonstrating a nonspecific character. However, the relatively slower rate of clearance of activity from the lungs of rats injected i.v., than from the

lower body of rats injected i.a., demonstrate a lung specific character as well. We also noted a tendency for liver-spleen activity to initially accumulate more slowly following i.a. infusion, as might be expected if an additional delay were occurring during passage through a extra microvascular bed before reaching this region (lower body to lung to liver-spleen). After 30 min, changes in regional activity did not significantly differ, and photographic images became nearly indistinguishable.

A previous study examined the activity remaining in excised lungs 30 min following i.v. and intraarterial infusion of leukocytes labeled with phosphorus-32 (^{32}P), at which time lung activity was noted to be comparable in the two groups (*31*). Lung activity was not examined prior to 30 min in that study. As discussed below, we did not examine the activity of excised organs in the current study, and therefore cannot make direct comparisons. However, we were not able to identify differences in lung activity from photographic images at 30 min in our study either (Fig. 2), and the clearance of activity from the lungs was not significantly different after 30 min. Therefore, our data appear consistent with that previous report.

As outlined in the methods section, we did not believe that we could reliably determine total lung activity from the images obtained with these small animals. Although we have previously reported whole lung activity in rats with use of a pinhole collimator, scatter from abdominal organs did appear to increase relative lung activity, when compared to excised lungs in that study (*11*). Because we could not simultaneously image a pair of rats with the pinhole collimator, we felt that scatter from liver-spleen images in the current study prohibited reliable estimation of activity in the immediately adjacent regions of the lung. Instead, we compared the rates of change in regional activity, normalized to maximal activity in that area, as has been reported by others (*8, 11*). Imaging the top of the lungs in supine rats, as well as the lower pelvis and thighs to reflect lower body, reduced the effect of scatter from liver and spleen images. These intraabdominal organs normally accumulate significant activity following ^{111}In-PMN infusions, as is evident in Figure 2.

The clearance of activity following i.v. infusion of ^{111}In-PMN in the current study was quite similar to that which we previously reported (*10*). Isolated organ activity examined in those previous studies has agreed with information obtained from images examined similarly (*10,11*). The current study was designed to examine transient, intravascular sequestration phenomena; therefore, demonstration of isolated organ activity from animals killed after the study period was not believed to add new or clearly relevant information to the current study.

A number of efforts to minimize nonphysiologic cell trauma in vitro have been reported which may decrease lung sequestration (*3,10,11,27–29*), including use of percoll-with-plasma for cell separation combined with tropolonate as the ligand for labeling (*8,20*). Although ficoll-hypaque was employed for cell separation in the current study, the methods used have been previously demonstrated to be associated with comparable in vivo performance characteristics (*3,4,10,11*) and in vitro function (*3,10*). Of interest, the use of hetastarch for vascular exchange permits harvest of sufficient numbers of PMN for labeling, without need to collect cells from an induced peritonitis or animals receiving toxins, which may alter cell functions (*10*). Although we cannot exclude the possibility that In-PMN sequestration might have been less using other methods, we believe that in vitro cell separation and labeling by any method is associated with some degree of cell stimulation. Therefore, the migratory patterns described in all labeled-cell studies likely reflect those of somewhat perturbed cells.

Cognizant of these issues, the current data are consistent with reports both of a selective and nonselective character of pulmonary sequestration of mobilized and potentially activated PMN. For example, preliminary data using histochemical techniques have demonstrated transient, pulmonary sequestration of unlabeled PMN mobilized from the bone marrow to a site of peripheral inflammation in rats (*30*). Likewise, stimulation of PMN in the venous circulation by polycellulose dialysis membranes (*22*), and i.v. injection of other polysaccharides such as zymosan and endotoxin (*23–24*), also are also associated with an accumulation of leukocytes in the lung. In contrast, one study reported that PMN sequestration was not pulmonary specific when cobra venom factor was infused intravenously (*25*). While our findings do demonstrate that nonpulmonary sequestration of In-PMN can occur as well, the relatively longer pulmonary transit time following i.v. infusion demonstrates differences in the transit of In-PMN through these microvascular beds.

While it is attractive to assume that the delayed clearance of activity from the lungs reflects adherence of cells to the endothelium, a number of other factors may affect the transit of PMN through these different microvascular beds: These include (a) the capacity to increase or divert blood flow through a region, including the recruitment of vessels, (b) cell deformability in microvascular beds where the luminal diameter is less than that of the nondeformed PMN, (c) the force with which blood flow pushes cells through these limiting microvascular beds and shears adherent cells from their walls, and (d) the interaction of PMN with their environment, including other cells such as endothelial cells. The current study was not designed to distinguish among these possibilities.

In conclusion, we have demonstrated a nonspecific

character to sequestration of In-PMN, as evident from the clearance of activity from the lower body following arterial infusion. However, a lung-specific character was also suggested by the relatively slower clearance of activity from the lung following intravenous infusion. The mechanisms responsible for this difference, and its potential physiologic role, remain to be identified. However, this lung specific character is consistent with the reports of marginated cells in the lung, which can then be recruited to the circulating pool (*32–33*). Additionally, the potentially greater exposure of the lung microvasculature to mediators released by circulating PMN which have been activated, may relatively localize PMN-mediated injury to the lung. The apparent lack of clinical injury following In-PMN infusion may reflect the limited degree of cell activation, and the relatively short duration of this potential interaction with the lung.

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