Induced Sputum Derives from the Central Airways
Confirmation Using a Radiolabeled Aerosol Bolus Delivery Technique

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Indirect evidence suggests that induced sputum derives from the surfaces of the bronchial Airways. To confirm this experimentally, we employed a radiolabeled aerosol bolus delivery technique that preferentially deposits aerosol in the central Airways in humans. We hypothesized that there would be significantly more radioactivity recovered in an induced sputum sample, and greater Airways clearance of radiolabeled particles, immediately after a central versus peripheral Airways deposition. Ten healthy volunteers underwent radiolabeled aerosol deposition (99mTc sulfur colloid particles) to the central and peripheral Airways on separate occasions followed immediately by induced sputum or no sputum (control), while seated in front of a gamma camera. Radioactivity was measured in the selected sputum sample, processed cell pellet, and supernatant fraction. Significantly more radioactivity was present in all portions of the sputum sample after central versus peripheral Airways deposition (i.e., selected sample: 15,607 counts ± 2,985 versus 943 counts ± 298, p = 0.001). Clearance from the whole lung was significantly greater 40 min after central versus peripheral Airways deposition (48 ± 3% versus 5 ± 1%, p = 0.0001). Compared with control, induced sputum greatly enhanced clearance after central deposition (48 ± 3% versus 11 ± 6%, p = 0.0001), but not after peripheral deposition (5 ± 1% versus 3 ± 0.8%). These results provide direct evidence that induced sputum derives from the central Airways with little or no contribution from the peripheral Airways.

Keywords: induced sputum; radiolabeled aerosol bolus

Induced sputum by inhalation of hypertonic saline has been used as a less invasive technique to examine Airways inflammation in healthy (1), allergic (2), and asthmatic individuals (3, 4). Indirect evidence supports the assumption that induced sputum selectively samples the surfaces of the bronchial Airways, while obtaining little or no secretions from the peripheral Airways. Studies that have compared induced sputum samples with bronchoalveolar lavage (BAL) in healthy subjects at rest demonstrate that sputum samples contain a significantly greater percentage of neutrophils, an observation considered to reflect Airways phenomena, whereas samples collected from Airways regions such as proximal bronchial secretions, tracheal secretions, and bronchial wash (BW) have similar leukocyte profiles to induced sputum (1, 5–8). We illustrated these observations pictorially using quantitative flow cytometry in a recent study (1). In that study, we also showed that macrophages and neutrophils recovered from induced sputum have modified surface phenotypes and functional capacity compared with the same cells from BAL (1), suggesting that these cells derive from different lung compartments.

Moreover, several studies have shown that induced sputum contains a higher concentration of fluid-phase components, such as eosinophil cationic protein (ECP), mucinlike glycoprotein, and albumin compared with BAL (6–9). These soluble components are thought to be present largely in Airways secretions compared with alveolar spaces. Finally, cough is a critical component in the sputum induction process. Clinical studies examining Airways clearance and deposition show that mucus clearance by cough is highly effective predominantly in the most proximal conducting (central) Airways (10). All these studies indirectly suggest that induced sputum selectively samples the central Airways, but there is no direct evidence to support this. Thus, the goal of this study was to directly test this hypothesis using quantifiable end points.

To achieve this aim, we used a novel aerosol generation and delivery system to examine Airways deposition and clearance in human volunteers (11–14). This system has recently been used to better characterize particle clearance kinetics from the conducting Airways by delivering small-volume (40-ml) aerosol boluses to shallow volumetric lung depths (i.e., less than 150 ml). We have further refined this technique to deliver jet-nebulized boluses of radiolabeled (99mTc) sulfur colloid (SC) particles within large droplets in order to target their deposition in the conducting Airways, hereafter referred to as central Airways. Clearance kinetics of these centrally deposited particles can also be compared with clearance from distal alveolar Airways, hereafter referred to as peripheral Airways, after large tidal volume inhalation of fine droplets containing the radiolabeled sulfur colloid particles. Using these novel aerosol delivery techniques, we hypothesized that if induced sputum derives primarily from the central Airways, this will be reflected as significantly greater radioactivity recovered in the sputum sample immediately after a central versus peripheral Airways deposition. Furthermore, if induced sputum is indeed an Airways phenomenon, clearance of radiolabeled particles from the central Airways should be markedly demonstrated immediately after a central Airways deposition. Likewise, little or no clearance should be observed immediately after a peripheral Airways deposition.

METHODS

Subjects

Ten healthy, nonsmoking volunteers 18 to 40 yr of age (4 male, 6 female) were recruited to participate in the study. All subjects received a medical examination on a separate screening day before beginning the study, and informed written consent was obtained. Female subjects provided a urine sample for pregnancy testing. A positive pregnancy test resulted in exclusion from the study. All subjects had no history of lung disease (asthma, fibrosis, chronic obstructive pulmonary disease) and had been free of upper or lower respiratory tract infections for 4 to 6 wk before beginning the study. All subjects had a forced expiratory volume in one second (FEV1) of greater than 80% of predicted values for a population of similar height, weight, sex, age, and race. Ethics approval for this study was obtained from the Committee on the Protection of the Rights of Human Subjects, School of Medicine, University of North Carolina–Chapel Hill.
Study Protocol

Each subject received four radiolabeled aerosol depositions (two peripheral and two central) on four separate occasions. Each radiolabeled particle exposure, whether central or peripheral, was approximated to be less than 20 μCi of the radioisotope technetium-99m (bound to SC) deposited in the lungs. Sputum induction was performed immediately after one central and one peripheral deposition. On each occasion, mucociliary clearance was measured over a 2-h period and at 24-h by gamma camera imaging (Elscint large-field-of-view, SP-4; Israel). A single-breath nitrogen washout test and a xenon-133 equilibrium scan (rebreathing 1 mCi/L) were performed on the first visit before an aerosol deposition to determine anatomic dead space (ADS) and effective lung volume imaging to normalize regional deposition, respectively (12). Single-breath nitrogen washout was measured from 500 ml above FRC according to the technique of Fowler (15). Subjects inhaled a single breath of 100% O2 from residual volume to FRC + 500 ml and exhaled again to residual volume (RV). A special Teflon mouthpiece designed to reduce the volume of the oral cavity was used for each individual. This same mouthpiece was used for the radiolabeled bolus inhalations described subsequently. The exhaled volumes associated with the ADS (exhaled volume through the midpoint of phase 2 of the nitrogen washout curve, as determined by the equal area technique [15]) were the average of three nitrogen washout curves for each subject. Next, subjects received a randomly assigned peripheral or central (bolus) aerosol deposition with or without sputum induction. After each aerosol deposition, subjects rinsed their mouths with water, and then sat with their back to the gamma camera for two initial 2-min scans. Immediately after these scans, subjects randomized to receive induced sputum underwent a modified induced sputum procedure while seated in front of the gamma camera.

After sputum induction, subjects remained in front of the gamma camera for an additional 1.5 h. Subjects who were not randomized to receive an induced sputum after their aerosol deposition remained seated in front of the gamma camera for 2-h after aerosol deposition. Subjects returned the following day for a 24-h postretention gamma camera image. At least 48 h later, subjects returned for their second visit period and received a randomized aerosol deposition with or without sputum induction. A total of four conditions completed the study: (1) peripheral deposition with induced sputum; (2) peripheral deposition without induced sputum; (3) central deposition with induced sputum; and (4) central deposition without induced sputum.

Central (Bolus) Airway Deposition Technique

An aerosol bolus is a discrete volume of air containing particles sandwiched within an inhaled volume of particle-free air. The depth to which the bolus penetrates into the lung is determined by the volume of the bolus and the volume of air inhaled after its insertion into the air stream. An injection at the beginning of an inspiration would preferentially deliver the aerosol to the periphery, whereas inhalation of the bolus at the end of a breath tends to deliver the aerosol to the extrathoracic and conducting airways. The inhalation is generally followed by a period of breath holding to maximize particle deposition on airway surfaces. In attempting to confine the aerosol to the ADS of the lungs, the boluses are small (< 50 ml) and delivered to shallow volumetric front depths (VFD), i.e., less than 150 ml (13, 14). The VFD is an estimate of the penetration of particles into the respiratory tract and represents the volume inspired from the point when the first particles enter the mouth to the end of inhalation (14). By normalizing the VFD to an individual’s ADS, the aerosol bolus may deposit more similarly in subjects of variable lung size.

On our central (bolus) airway deposition study days, a small bolus (40 ml) was delivered to shallow VFDs in the lung. Radiolabeled (99mTc) SC (50 mCi) was prepared using TechneScan Sulfur Colloid Kits (Mallinckrodt Medical, Maryland Heights, MO) (16). The binding of 99mTc to SC was always greater than 99% determined by paper chromatography. The 99mTc-SC particles in solution are submicron with a number mean diameter of 0.22 μm and geometric standard deviation of 1.75. The solution was then placed in a modified DeVilbiss 646 jet nebulizer, which generated 6 μm mass median aerodynamic diameter (MMAD) polydisperse (when pulsed at 40 psi), i.e., aqueous particles containing the smaller suspended 99mTc-SC particles. In this way, the 99mTc-SC particles were deposited as 6-μm droplets but dissociated into 0.22-μm particles. By computer-controlled activation of the compressed air source used to disperse the nebulizer solution, the boluses were delivered to a VFD of 0.6 ADS for each subject. After inhalation of each bolus under controlled conditions (500 mCi tidal volume at 125 mls flow), the subject held his or her breath for 5 s followed by a rapid exhalation to maximally deposit the particles on the conducting airways. Also during the bolus inhalations the relative aerosol concentration and respired volumes were measured by photometry and a pneumotachograph at the mouth to determine the VFD of each inhaled bolus. In general, inhalation of 20 to 30 boluses over a 15-min period was required to deliver sufficient activity to the lungs (approximately 15 μCi), as monitored by a single-crystal NaI detector placed at the subject’s back.

Peripheral Airway Deposition Technique

To deliver particles to the peripheral, alveolar region of the lung, we nebulized radiolabeled SC solution with an Ultravent nebulizer (Mallinckrodt Medical, which produced a mean droplet size of 0.7 μm). Subjects inhaled this aerosol while matching a 1-L tidal volume, 12 breaths/minute breathing pattern displayed on an oscilloscope. The combination of this slow, deep breathing pattern and the small particle size was chosen to maximize alveolar deposition relative to that in the bronchial airways (17, 18). The subjects inhaled the radiolabeled aerosol until approximately 15 to 20 μCi were deposited in their lungs (< 2 min), again as monitored by the single-crystal NaI detector placed at their backs.

Gamma Camera Analysis

To assess the degree of central (C) versus peripheral (P) airway deposition within the lung for all four study days in each subject, we calculated a C/P ratio of 99mTc activity, normalized to the xenon-133 equilibrium scan, on the initial deposition scan after radiolabeled aerosol inhalation (12, 19). This normalization was done to account for the difference in relative lung areas and thickness between the central and peripheral lungs. The central and peripheral regions of interest (ROI) (solid line) is shown for both lungs (area = 25% of the whole lung ROI). Greater deposited activity is shown in red. The ROI for the stomach is shown in the bottom quadrant of the left lung to delineate and subtract stomach activity.
ripheral regions. Whereas both the central and peripheral regions (Figure 1) overlay alveoli and small airways, the central region also incorporates large, bronchial airways not present in the peripheral region. Thus, increases in C/P to values greater than 1.0 reflect increased large airway deposition. To eliminate activity associated with the stomach in the left P region, it was necessary to exclude the lower left lung base when creating the left lung region of interest (Figure 1, lower left region). This should have had no effect on our C/P comparisons because the same regions were used for both central and peripheral deposition days and the C/P was normalized to associated lung volumes ($^{133}$Xe equilibrium) in each case. A rectangular region bordering the right and left lung (defined by the $^{133}$Xe equilibrium scan) was used to determine, by computer analysis, the whole lung clearance as a percentage of the initial counts (background-corrected and decay-corrected) over the gamma camera scanning period of 2 h and at 24 h. Again, care was taken to ensure that the activity in the stomach was excluded from the left lung region.

**Induced Sputum**

*Sputum induction and collection.* Induced sputum samples were collected and measured for radioactivity at 40 min after aerosol deposition. We followed the method published by Pin and coworkers (20) with some modifications. In brief, forced expiratory volume in one second (FEV$_1$) and vital capacity (VC) were measured before saline inhalation to determine baseline values. The FEV$_1$ values that matched a 20% fall from baseline were determined and recorded. An ultrasonic nebulizer was filled with 30 ml of 5% hypertonic saline (NaCl) for the first of two 12-min inhalations. During each inhalation period, the subject was encouraged to come off the mouthpiece at any time to cough if a sputum sample from the lower airways (i.e., not from the back of the throat) was ready for expectoration. A separate sterile container designated as waste was used to collect saliva–salt water that collected in the mouth and throat during the inhalation periods. After the 12-min inhalation period and before expectoration, subjects underwent a three-step cleansing procedure: (1) Gargle and rinse the mouth with water. (2) Scrape and clear the back of the throat (to avoid the inclusion of nonairway fluid samples), and then expectorate this into the sink. (3) Blow one’s nose. Next the subjects delivered a chesty cough and expectorated the secretions into a sterile specimen jar, which was capped and placed on ice. Each subject coughed as many times as he or she felt necessary in order for airway secretions to be expectorated. This was typically 5 to 7 cough attempts. A postinhalation measurement of FEV$_1$ was then performed. Provided the FEV$_1$ decreased by less than 20% from the baseline value, the subjects repeated the second 12-min inhalation period with 5% saline followed by the cleansing procedure and cough attempt.

**Sputum Analysis**

Immediately after sputum induction, the expectorated sample and waste container were weighed and taken to a scintillation counter (Nuclear Data Sodium Iodide [NaI] Scintillation Counter; Smyrna, GA) to determine the level of radioactivity (counts) present in each. The sputum sample was transferred to a sterile Petri dish and its characteristics (including squamous epithelial cells) and cell viability. Particles. The sample was gently aspirated with a pipette and agitated (bench rocked) for 15 min. A volume of Dulbecco’s phosphate-buffered saline (D-PBS) equal to 4× the sample weight was added to the sample, and the sample was bench rocked for 5 min.

The sample was then filtered through a 48- to 52-μm diameter nylon mesh and the weight of the cell suspension was recorded. After this, the sample was centrifuged (1,000 rpm, 5 min) and the supernatant collected into a separate tube. A separate experiment also showed that this level of centrifugation was not capable of spinning down the “free” SC particles in solution. The level of radioactivity was measured in the cell pellet and supernatant. Next, a Neubauer hemocytometer and trypan blue exclusion staining (1:1 dilution of sample:trypan blue) were used to determine the total cell count (excluding squamous epithelial cells) and cell viability.

**Normalization of Sputum Radioactivity to Deposited Radioactivity**

The radioactivity measured in the sputum samples by the scintillation detector was also dependent on the total amount of activity deposited in the lung for either the central or peripheral depositions. It was therefore necessary to normalize these sputum counts in order to make both intrasubject and intersubject comparisons. This normalization was accomplished as follows:

\[
\text{NRC (normalized radioactive counts) =} \\
\frac{\text{Measured counts} \times \text{whole lung gamma camera counts for specific subject and study day}}{\text{average whole lung gamma camera counts for all subjects on both central and peripheral sputum induction study days}}
\]

The whole lung gamma camera counts were determined from the initial deposition image (i.e., Figure 1) (before beginning the sputum induction procedure) and the rectangular regions bordering the left and right lungs. For each subject and study day, the measured counts were first background-corrected and decay-corrected ($^{99m}$Tc has a 6-h half-life) to the time of the initial gamma camera deposition image.

**Statistical Methods**

Statistically significant differences between multiple study end points were assessed using a repeated-measures one-way analysis of variance.

**TABLE 1. ANTHROPOMETRIC DATA**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (yr)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>Smoker</th>
<th>FEV$_1$ (l)</th>
<th>FVC (l)</th>
<th>% Pred FEV$_1$</th>
<th>% Pred FVC</th>
<th>ADS (ml)</th>
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<tbody>
<tr>
<td>F</td>
<td>34</td>
<td>160</td>
<td>64</td>
<td>No</td>
<td>2.63</td>
<td>3.55</td>
<td>92</td>
<td>106</td>
<td>150</td>
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<td>F</td>
<td>26</td>
<td>173</td>
<td>62</td>
<td>No</td>
<td>3.42</td>
<td>4.19</td>
<td>100</td>
<td>104</td>
<td>150</td>
</tr>
<tr>
<td>M</td>
<td>50</td>
<td>190</td>
<td>105</td>
<td>No</td>
<td>5.40</td>
<td>6.73</td>
<td>116</td>
<td>117</td>
<td>270</td>
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<tr>
<td>F</td>
<td>52</td>
<td>157</td>
<td>82</td>
<td>No</td>
<td>2.36</td>
<td>2.67</td>
<td>116</td>
<td>108</td>
<td>130</td>
</tr>
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<td>M</td>
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<td>172</td>
<td>77</td>
<td>No</td>
<td>4.74</td>
<td>6.15</td>
<td>191</td>
<td>102</td>
<td>170</td>
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<td>M</td>
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<td>93</td>
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<tr>
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<td>173</td>
<td>84</td>
<td>No</td>
<td>3.30</td>
<td>4.07</td>
<td>80</td>
<td>85</td>
<td>170</td>
</tr>
<tr>
<td>F</td>
<td>21</td>
<td>168</td>
<td>67</td>
<td>No</td>
<td>3.46</td>
<td>3.87</td>
<td>121</td>
<td>117</td>
<td>130</td>
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<tr>
<td>F</td>
<td>23</td>
<td>163</td>
<td>73</td>
<td>No</td>
<td>3.11</td>
<td>3.42</td>
<td>115</td>
<td>110</td>
<td>160</td>
</tr>
<tr>
<td>F</td>
<td>20</td>
<td>168</td>
<td>61</td>
<td>No</td>
<td>3.12</td>
<td>3.64</td>
<td>108</td>
<td>109</td>
<td>130</td>
</tr>
<tr>
<td>Average</td>
<td>31.2</td>
<td>173.9</td>
<td>76.8</td>
<td></td>
<td>3.56</td>
<td>4.43</td>
<td>103</td>
<td>106</td>
<td>160</td>
</tr>
</tbody>
</table>

**SEM**

|        | 4.0    | 13.7        | 4.6         |        | 1.12       | 1.39   | 4             | 3         | 10     |

*Definition of abbreviation: ADS = anatomic dead space.*
Table 2. Total and Differential Cell Counts from Induced Sputum*

<table>
<thead>
<tr>
<th>Aerosol Deposition</th>
<th>Total Sputum Sample (mg)</th>
<th>Total Selected Sputum Sample (mg)</th>
<th>Total Cells (10⁶)</th>
<th>% PMN</th>
<th>% Mac</th>
<th>% Lym</th>
<th>Cell Viability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central</td>
<td>7,272 ± 736</td>
<td>3,446 ± 448</td>
<td>3.65 ± 0.5</td>
<td>23.3</td>
<td>75.7</td>
<td>1.14</td>
<td>73.44 ± 3.9</td>
</tr>
<tr>
<td>Peripheral</td>
<td>7,555 ± 1,020</td>
<td>2,663 ± 434</td>
<td>3.819 ± 1.0</td>
<td>15.5</td>
<td>83.5</td>
<td>1.28</td>
<td>60.0 ± 5.9</td>
</tr>
</tbody>
</table>

Definition of abbreviations: Lym = lymphocytes; Mac = macrophages; PMN = polymorphonuclear neutrophils.
* Values are mean ± SEM.
† Differential cell counts are % nucleated cells excluding squamous epithelial cells. Mean % squamous epithelial cells were 10.1% (central) and 16.3% (peripheral).

Results

Table 1 shows anthropometric data for all subjects (4 male, 6 female). Each subject met the spirometric inclusion criteria of ≥ 80% of predicted baseline values for FEV₁ and FVC. Mean (SEM) group values for percentage of predicted FEV₁ and FVC were 103 and 106%, respectively.

No significant differences (p < 0.05) were observed in total and differential cell counts between central and peripheral aerosol depositions. Satisfactory sputum samples were obtained from all subjects on all occasions (i.e., each selected sputum sample from all subjects on all occasions contained greater than 1,000 mg of selected sample).

Figure 2 shows the mean ± SEM number of NRC recovered in the total expectorated sputum sample, the manually selected portion of the total expectorated sample, and the nonprocessed waste (saliva generated from the induction process) for the central deposition. A minimal number of NRC was present in the waste component (i.e., waste/total = 4.8%), reflecting a negligible contribution of counts owing to deposition in the mouth and oropharynx during the deposition procedure. In contrast, a high percentage of NRC was recovered in the selected portion of the sputum sample (i.e., selected/total = 86%), indicating that the majority of radiolabeled particles were associated with sputum plugs.

Discussion

The widespread use and growing popularity of induced sputum as a means of examining airways inflammation in human volunteers has made it important to confirm experimentally the location from which the sputum sample derives. Several studies already suggest that induced sputum selectively samples the central airways (1–5). These studies performed comparative leukocyte analyses on induced sputum, BAL, and bronchial secretions and showed greater neutrophil proportions in both sputum and bronchial samples compared with BAL. Although these data provide useful information to support the premise that sputum is an airways phenomenon, they do not necessarily test this hypothesis.

In this study, we used a novel radiolabeled aerosol bolus delivery system that yielded more quantifiable data to show that induced sputum selectively samples the central airways with little or no contribution from the peripheral airways. The success of the bolus technique to deposit relatively more particles in the central versus peripheral airways was evident in two
ways. First, the C/P ratio was greater than 1 and significantly increased for the central versus peripheral deposition, implying greater deposition in all the bronchial airways, because deposition at the level of generations 1 to 3 will not likely occur independent of enhanced deposition throughout the rest of the conducting airways (generations 4 to 16). Second, we found that with the central deposition maneuver, approximately 60% of the particles cleared by 24 h (with or without sputum induction, Figure 5A), whereas with the peripheral deposition maneuver we only had approximately 20% clearance of particles through 24 h. From our own work (12), it is reasonable to assume that percentage of clearance through 24 h is an estimate of particles initially depositing in the conducting airways. Consequently, this represents a 3-fold greater particle deposition in conducting airways for the central versus peripheral maneuver. In addition to successfully applying the bolus technique in this study, we also considered whether any potential biases occurred in the concentration of deposited aerosol between peripheral and central airways owing to the possible differences that may be present in airways surface liquid volume. Based on estimates from the literature of airways surface liquid volume depth (10 to 30 μm) (21) and airways surface area (6,300 cm²) (22), we calculated the airways surface liquid volume to be 6.3 ml compared with a morphologically estimated alveolar fluid volume of 7.15 ml (23, 24). These limited data suggest that volumes of airways surface liquid may not differ that much between airway and alveolar regions. Consequently, there should be minimal bias associated with variable airways surface liquid volumes in central and peripheral regions.

When we compared the level of radioactivity recovered in the selected sputum sample after a central versus peripheral airways deposition, we observed a marked increase in the number of NRC recovered in all portions of the selected sample (total, cell pellet, supernatant) after a central airways deposition. The NRC in the selected portion represented 86% of the total sample, whereas its weight was less than 50% of the total. This indicates that particles were concentrated in the selected portion of the

Figure 3. Mean (SEM) NRC recovered in the total selected sputum sample, cell pellet, and supernatant fraction immediately after deposition of radiolabeled aerosol in central versus peripheral airways in healthy individuals (n = 10). Significantly more radioactivity was present in all sample portions after the central deposition (filled bar) versus peripheral (open bar) deposition (*p = 0.001). *Significantly different from central deposition (p = 0.001).

Figure 4. (A) Mean (SEM) whole lung clearance of radiolabeled particles with induced sputum after central and peripheral airways deposition in healthy individuals (n = 10). Significantly greater clearance was observed at 40 min, 120 min, and 24 h for sputum induction after a central (dark bar) and peripheral (clear bar) airways deposition (*p = 0.0001). (B) Mean (SEM) C/P ratios for the central and peripheral airway deposition in healthy individuals (n = 10). Significantly greater C/P ratios were observed after the central (filled bar) versus peripheral (open bar) airways deposition (*p = 0.0005).
sputum sample and not simply due to selecting 86% of the total sample weight. Furthermore, there were minimal (yet detectable) levels of NRC present in the sputum samples after the peripheral deposition. If induced sputum did in fact sample the peripheral airways to any significant degree, we clearly would not have observed as great a magnitude difference in particle recovery between the central and peripheral airway depositions. The detection of some radioactive counts after the peripheral deposition reflects the fact that a minimal amount of aerosol was deposited in the bronchial airways during the peripheral deposition condition. This is supported by the fact that the bulk of radioactive counts from the total sputum samples (either on central or peripheral study days) were associated with the selected “plug” sample, and within that, most of the particles were associated with the supernatant compared with the cells. The supernatant is likely to contain the mucous gel portion of airway secretions, and because mucins primarily derive from glandular and goblet cell secretions of conducting airways, these data provide further evidence that the total sputum samples after central and peripheral deposition are derived from the same conducting airways. Moreover, because we observed a significantly greater C/P ratio and a greater percent clearance at 24 h for the central versus peripheral deposition, we were assured that the bolus aerosol deposition more selectively targeted the central airways relative to the peripheral airways, and hence established valid central and peripheral deposition conditions.

The measurement and comparison of clearance kinetics after a central and peripheral airways deposition also provided good evidence of selective central airways sampling by induced sputum (Figure 4A). Previous studies have shown that inhalation of hypertonic saline, or cough alone, can enhance clearance of deposited particles from the lung when compared with clearance without hypertonic saline or clearance without cough. However, these previous studies (25, 26) did not attempt to distinguish between centrally (airway) and peripherally (alveolar) deposited particles when assessing the effects of hypertonic saline or cough. After sputum induction, we observed 50% clearance at 40 min for a central deposition, compared with a negligible 5.0% clearance for a peripheral deposition. This is consistent with previous reports that little or no aerosol is expected to be cleared at 40 min after a peripheral airway deposition (18, 19). Because induced sputum employs the combination of both hypertonic saline inhalation and cough, it may not have been surprising that we demonstrated greatly enhanced clearance, especially for our central deposition pattern, compared with the no sputum control condition, considering that cough is most effective in the large conducting airways, where shear rates are highest (greatest air velocities) and the depth of airway surface fluid is likely greatest (27). Finally, as further evidence that the sputum induction technique does not sample the deep lung, the 24-h retention (i.e., 100 – % 24-h clearance, Figures 5A and 5B) was unchanged for both central and peripheral depositions between the sputum and no sputum induction study days. These data further confirm our hypothesis that induced sputum samples derived from the central airways, not the alveolar regions of the lung.

For the central deposition sample, the average ratio of activity in the cell pellet (i.e., cell-associated particle ratio) versus the sum of the cell pellet plus supernatant was 12 ± 3%, if we considered only those individuals for whom we recovered more than 80% of total sputum activity in the cell pellet plus supernatant. Similar radiolabeled aerosol studies performed in animal models (hamster macrophages) show a higher cell-asso-

![Figure 5](image-url)
References