Am J Physiol Lung Cell Mol Physiol 286: L1002–L1008, 2004.
First published December 24, 2003; 10.1152/ajplung.00260.2003.

Alveolar macrophages are a primary barrier to pulmonary absorption of macromolecules

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Submitted 29 July 2003; accepted in final form 22 December 2003

Lombry, Catherine, David A. Edwards, Véronique Préat, and Rita Vanbever. Alveolar macrophages are a primary barrier to pulmonary absorption of macromolecules. *Am J Physiol Lung Cell Mol Physiol* 286: L1002–L1008, 2004. First published December 24, 2003; 10.1152/ajplung.00260.2003.—We demonstrate that a primary source of elimination of inhaled macromolecules after delivery to the lungs and before absorption into the systemic circulation owes to clearance by alveolar macrophages (AM). Depletion of AM by liposome-encapsulated dichloromethylene diphosphonate is shown to cause severalfold enhancement in systemic absorption of IgG and human chorionic gonadotropin after intratracheal instillation in rats. Lowering the doses of IgG delivered to the lungs alleviates local degradation and results in a dramatic increase in systemic absorption of the protein as well. Chemical and physical means of minimizing uptake of macromolecules by AM are proposed as novel methods for enhancing protein absorption from the lungs. Such strategies may have important ramifications on the development of inhalation as an attractive mode of administration of therapeutic proteins to the bloodstream.

pulmonary drug delivery; protein degradation; absorption enhancement

INHALATION AEROSOLS OFFER significant potential for noninvasive systemic administration of peptide and protein therapeutics (1, 23). Yet, low bioavailabilities result from uncontrolled biological losses of molecules in the airway lumen and/or respiratory tissue and considerably diminish efficiency (23). Insulin (5.8 kDa) inhaled into the human lungs using high technology inhaler devices or engineered particles absorbs into the bloodstream within minutes and with an average bioavailability of 10% relative to subcutaneous injection (5, 23, 27). Large proteins, like IgG (150 kDa), absorb into the bloodstream after intratracheal instillation in animals over many hours and with bioavailabilities that can be significantly lower than 5% (9, 16).

The exact nature of protein absorption into the bloodstream and elimination from the lungs is unclear, although it appears certain that most protein absorption occurs in the large and highly vascularized alveolar region, with the alveolar epithelium playing a key role in regulating protein passage into the bloodstream (8, 25, 30, 32). It has long been assumed that the primary obstacles to an efficient absorption of inhaled proteins reside in the barrier property of the alveolar epithelium and local proteases (8, 25, 30, 32). Therefore, strategies to increase systemic absorption of macromolecules from the lungs over the last decade have primarily involved membrane permeation enhancers (such as bile salts) that act to better permeabilize the lung's epithelium or protease inhibitors that neutralize local enzyme activities (14, 15, 18).

We demonstrate that alveolar macrophages (AM) comprise a major "barrier" to the transport of macromolecules from the lungs into the bloodstream, particularly for moderate-sized to large proteins. Using intratracheal instillation of liposome-encapsulated dichloromethylene diphosphonate (Cl_2MDP), we depleted rat lungs of AM and thereby assessed their role in the pulmonary fate of proteins in vivo. Severalfold enhancement in systemic absorption of immunoglobulins and human chorionic gonadotropin (hCG) from the lungs followed the elimination of AM. Our studies suggest a novel means for enhancing the efficiency of protein absorption from the lungs, i.e., lowering AM uptake of macromolecules by chemical or physical means. For certain proteins, such as immunoglobulins, diminishing the total delivered dose also provides a simple and efficient physical method to diminish degradation and dramatically favor systemic absorption of the macromolecule from the lungs.

MATERIALS AND METHODS

Materials. Rat IgG1 anti-dinitrophenyl hapten was purchased from LO-IMEX (Brussels, Belgium), hCG (Profasi) was purchased from Serono Benelux (Brussels, Belgium), and human insulin was purchased from ICN Biomedicals. Dinitrophenyl human albumin, Cl_2MDP, FITC isomer I, FITC-insulin from bovine pancreas (90%), sulforhodamine, sodium xylazine, sodium cacodylate, and cholesterol were supplied by Sigma (Bornem, Belgium). Lecithin was kindly donated by Lipoid (Ludwigshafen, Germany). Horseradish peroxidase-conjugated anti-rat IgG Fc region was obtained from Pharmingen (Becton Dickinson Biosciences; Erembogem, Belgium). Pentobarbital sodium and ketamine (Ketalar) were purchased from Certa (Medeva Pharma, Braine-l'Alleud, Belgium) and Warner-Lambert (Zaventem, Belgium), respectively. Formaldehyde was obtained from Merck Eurolab (Leuven, Belgium), and glutaraldehyde was from Fluka (Neu-Ulm, Switzerland). Hanks' balanced salt solution without Ca^{2+}, Mg^{2+}, and phenol red was purchased from Life Technologies (Merelbeke, Belgium).

Animals. Twelve-week-old male Wistar rats were supplied by Elevage Janvier (Le Genest St Isle, France) and used within 3 wk of delivery for confocal laser scanning microscopy studies and within 1 wk for pharmacokinetic studies. Animals had free access to tap water and laboratory diet (pelleted commercial standard diet no. A04; Usin Alimentation Rationnelle, Epinay-sur-Orge, France) and were kept on a 12-h day-night cycle until they were used. Rats were anesthetized with ketamine/xylazine (90/10 mg/kg) intraperitoneal injection before intratracheal or intravenous (IV) injections as well as before lung

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preparation for analysis by confocal laser scanning microscopy. Slighter anesthesia was given before orbital bleeding. All experimental protocols in rats were approved by the Institutional Animal Care and Use Committee of the Université catholique de Louvain.

Confocal laser scanning microscopy. Rat IgG1 anti-dinitrophenyl hapten and hCG were custom labeled with FITC (19). FITC-IgG, FITC-hCG, and FITC-insulin were localized by confocal laser scanning microscopy in the pulmonary tissue after intratracheal instillation in rats, as previously described (19). Briefly, 500 μg of FITC-IgG, 100 μg of FITC-hCG, or 40 μg of FITC-insulin (dissolved in 100 μl of NaCl 0.9%) were instilled in the rat lungs using a Microsprayer device (PennCentury, Philadelphia, PA) inserted in the trachea via the mouth. Within 1 min and at intervals up to 4 days after intratracheal delivery, the lungs were lavaged and fixed by vascular perfusion of PBS (pH 7.4) containing 0.1% sulforhodamine for 5 min and then a fixative solution (0.6% formaldehyde, 0.9% glutaraldehyde, 75 mM sodium cacodylate, and 0.1% sulforhodamine, adjusted to pH 7.4) for an additional 5 min. The thoracic cavity was opened, and the lungs were removed for analysis by confocal laser scanning microscopy. The total time for intratracheal delivery and lung preparation for microscopy was ~15 min. Each experimental condition was repeated at least three times.

Slices (± 2 mm) of the lobes and trachea were placed directly in a sample holder and covered with a coverslip glass. The confocal microscope was a Bio-Rad MRC 1024 confocal unit equipped with an argon-krypton laser and mounted on a Zeiss Axiovert 135M inverted microscope. Laser excitation wavelengths of 488 and 568 nm were used individually to scan lung tissue, and fluorescent emissions from FITC (emission λ = 515–545 nm) and sulforhodamine (emission λ = 589–621 nm) were collected using separate channels. Images were acquired with a Zeiss Plan-Neofluor ×40 oil immersion and ×10 lenses. Grayscale images (obtained from each scan) were pseudocolored green (FITC-IgG, FITC-hCG, or FITC-insulin) and red (sulforhodamine) and then overlaid (Zeiss LSM confocal software) to form a multicolored image.

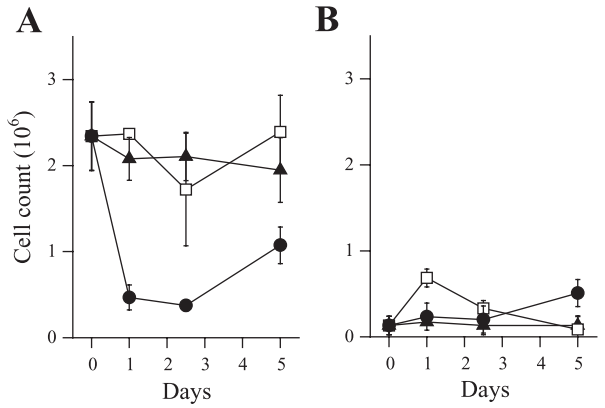

Fig. 2. Impact of intratracheal instillation of Cl_2MDP liposomes on the number of alveolar macrophages (*A*) and neutrophils (*B*) in bronchoalveolar lavage. Rats were intratracheally instilled with 750 μl of 0.9% NaCl (▲, n = 3), PBS liposomes (□, n = 3), or Cl_2MDP liposomes suspension (●, n = 3). Bronchoalveolar lavages were performed before and 1, 2.5, and 5 days after instillation. Values are means ± SE.

The uptake of FITC-IgG by AM was further visualized by analyzing AM collected by bronchoalveolar lavage (BAL). The rats were killed with an overdose of pentobarbital 2 h after FITC-IgG delivery. The airways and lungs were washed with Hanks' balanced salt solution without Ca^{2+}, Mg^{2+}, and phenol red to minimize cell clumping. The BAL was obtained by slowly injecting 10 ml of Hanks' balanced salt solution into the trachea, waiting for 30 s, and then withdrawing the liquid from the lungs. The lavage procedure was repeated with four additional 10-ml aliquots until a total volume of 50 ml was injected. The BAL was centrifuged at 700 *g* and 4°C for 10 min. The supernatant was removed, and the cells were resuspended in 1 ml of Hanks' balanced salt solution. A few droplets of the cell

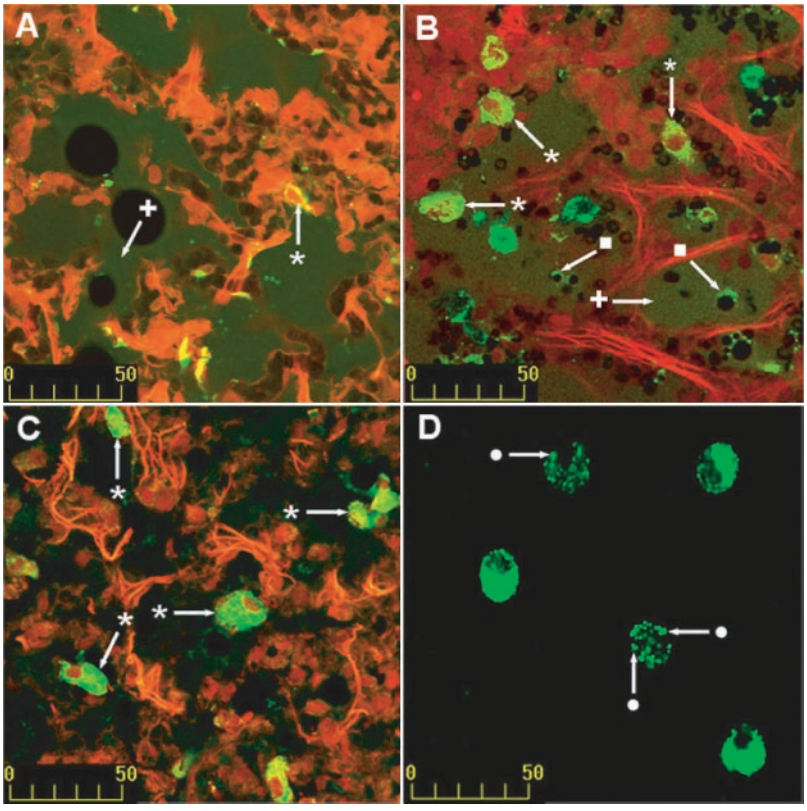

Fig. 1. Localization of FITC-IgG in rat lungs by confocal imaging. The alveoli were visualized 15 min (*A*), 24 h (*B*), or 3 days (*C*) after intratracheal instillation of a FITC-IgG (green) solution (500 μg in 100 μl of 0.9% NaCl) to the lungs of rats in vivo. Tissue was colored red with sulforhodamine. Arrows indicate alveolar macrophage (AM) loading FITC-IgG (*), soluble FITC-IgG in the alveolar fluid (+), and some particulate FITC-IgG in the air spaces (■). Intensely fluorescent endocytosis vesicles (●) are observed in the cytoplasm of AM recovered by bronchoalveolar lavage 2 h after instillation of the FITC-IgG solution (*D*). Scale bars are 50 μm.

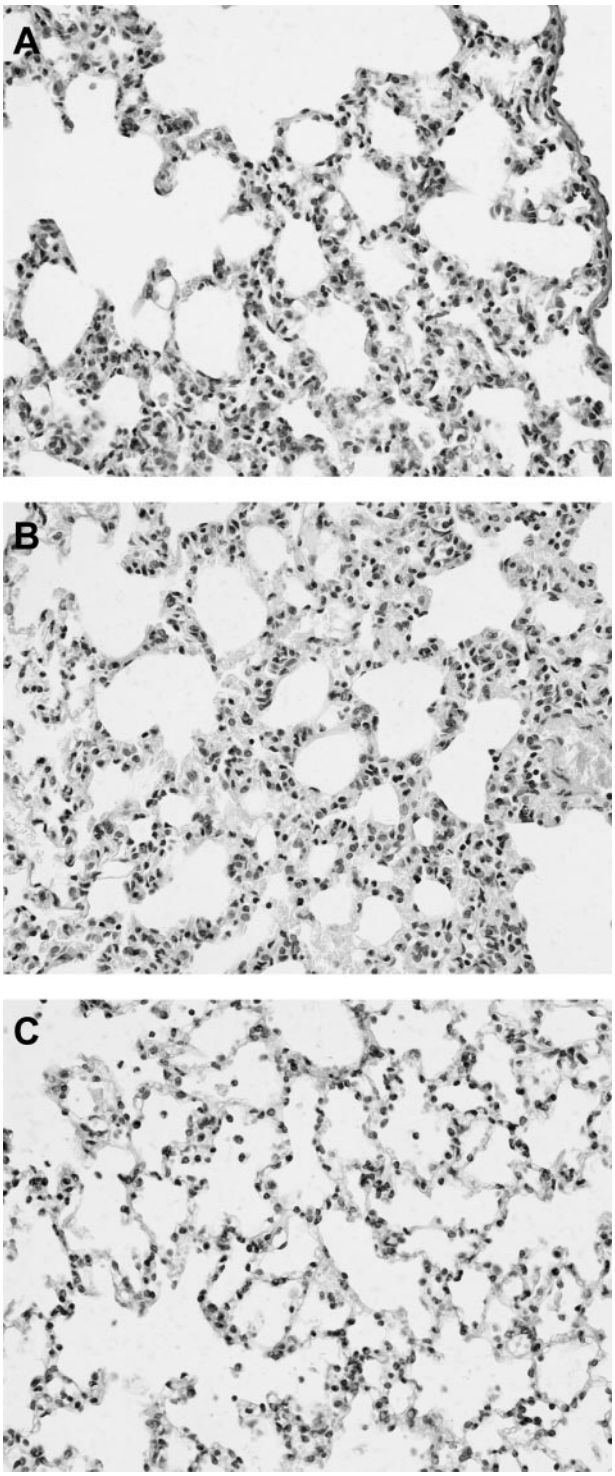

Fig. 3. Photomicrographs of eosin/hematoxylin-stained lung sections 24 h after intratracheal instillation of 0.9% NaCl (*A*), PBS liposomes (*B*), or Cl_2MDP liposomes (*C*) suspension. Magnification, ×20.

suspension were placed directly in a sample holder and covered with a coverslip glass for analysis by confocal laser scanning microscopy.

To assess the autofluorescence properties of the pulmonary tissue, samples were examined with the confocal microscope in the absence of administration of fluorescent markers. The autofluorescence of the rat lung in the green channel was found to be very low with the confocal settings used in this study, except in peripheral regions where fiber networks and cells exhibited high autofluorescence (data not shown). To avoid these areas of high-fluorescence background, confocal images were collected only in regions at least 10 μm below lung surface (19).

Preparation of Cl_2MDP and PBS liposomes. Eighty-six milligrams of lecithin and 10 mg of cholesterol were dissolved in 10 ml of chloroform. A lipid film was produced by low-vacuum rotary evaporation, and the film was dispersed by gentle rotation in 10 ml of PBS or 10 ml of Cl_2MDP solution (2.5 g/10 ml of sterile water). After the film was completely removed, the suspension was kept for 30 min at room temperature under nitrogen atmosphere, and the suspension was sonicated for 2 min at a power of 60 W (Sympatec, Clausthal-Zellerfelg, Germany). Free Cl_2MDP was removed by dilution of the suspension in 100 ml of PBS and centrifugation at 10,000 rpm for 30 min. The liposome pellet was resuspended in 1.5 ml of PBS. The suspension of Cl_2MDP or PBS liposomes was stored under nitrogen atmosphere at 4°C for a maximum of 1 wk (29).

Depletion or IT instillation of AM. Seven hundred and fifty microliters of PBS or Cl_2MDP liposome suspension or of 0.9% NaCl were instilled in the trachea of rats using a syringe inserted via the mouth. AM depletion resulting from Cl_2MDP was assessed by analyzing cellular components in BAL before and 1, 2.5, and 5 days after liposome or saline delivery. The total number of cells was determined by mixing with Turch liquid and counting with a hemacytometer. AM were differentiated from neutrophils on cytocentrifuge preparations fixed in methanol and stained with Diff-Quik (300 cells/rat; Dade Berhing, Düdingen, Switzerland). Lung histology was used to assess the integrity of the airway and alveolar epithelia after instillation of PBS or Cl_2MDP liposomes. Rats were killed 24 h after treatment of the lungs with liposomes. The lobes and trachea were removed and placed in a 4% formol solution. Paraffin-embedded histological sections were stained with hematoxylin/eosin and examined by light microscopy.

An increase in the number of AM present locally in the lungs was achieved by instilling additional AM into the lungs of intact rats. AM

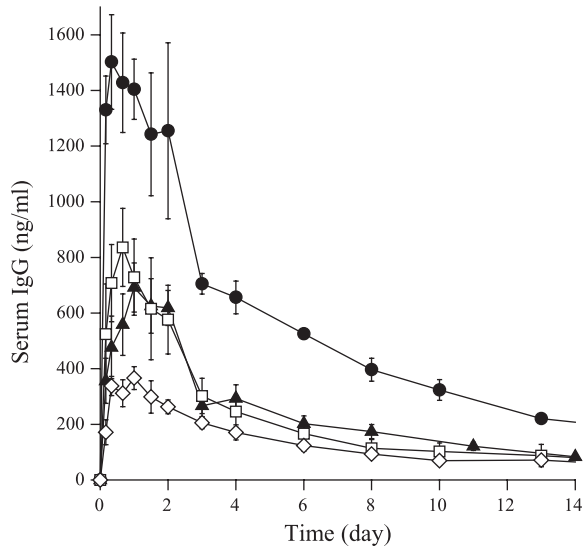

Fig. 4. Pharmacokinetics of pulmonary IgG in rats with variable numbers of AM. Rats were depleted from AM using Cl_2MDP liposomes (●, $n = 4$), were intratracheally instilled with PBS liposomes (□, $n = 4$) or additional AM (◇, $n = 5$), or remained untreated (▲, $n = 6$). One day after liposome treatment, 1 h after instillation of AM, or no treatment, rats received an IgG dose of 500 μg by intratracheal instillation. The bioavailability of IgG increased from 4.7 ± 1.1% (untreated rats) or 4.5 ± 2.5% (PBS liposomes) to 10.5 ± 3.5% (Cl_2MDP liposomes) and conversely decreased to 2.6 ± 1.0% (additional AM instilled) compared with an intravenous dose of 50 μg (presented in Fig. 8*B*). Values are averages ± SE.

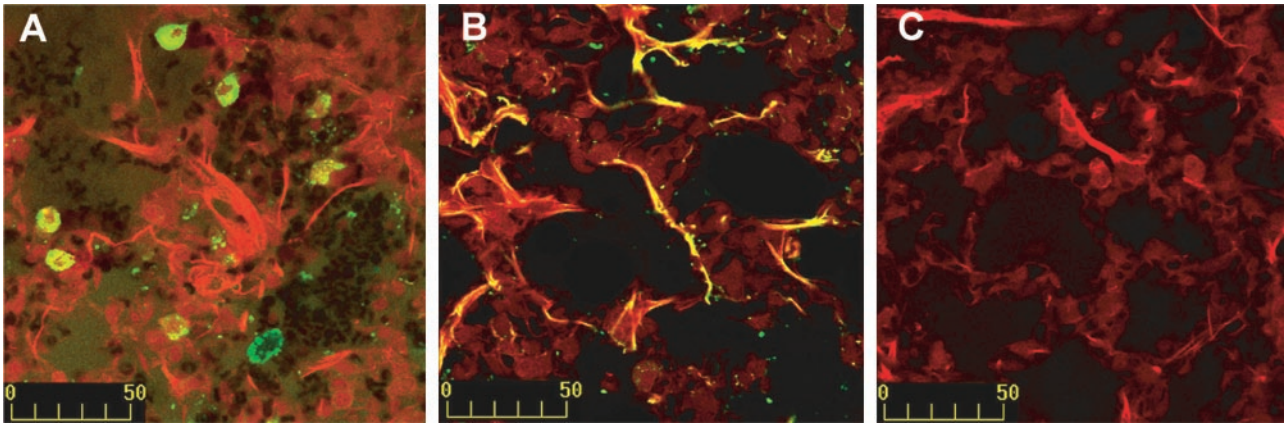

Fig. 5. Localization of FITC-human chorionic gonadotropin (hCG) and FITC-insulin in rat lungs by confocal imaging. Alveoli were visualized 6 h after intratracheal instillation of a FITC-hCG (green) solution (100 μg in 100 μl of 0.9% NaCl; *A*) or 30 min (*B*) or 6 h (*C*) after intratracheal instillation of a FITC-insulin solution (40 μg in 100 μl of 0.9% NaCl) to the lungs of rats in vivo. Tissue was colored red with sulforhodamine. Scale bars are 50 μm.

were collected by BAL, the suspensions of AM from several rats were pooled, and five million cells (a BAL from 1 rat yields an average of 2.5 million AM) suspended in 200 μl of Hanks' balanced salt solution were intratracheally instilled into the lungs of each intact animal. It is noteworthy that five million AM represent only ~20% of the total AM population in rats (28).

Pharmacokinetic studies. Rats (413 ± 21 g) were intratracheally instilled with liposomes or additional AM 1 day or 1 h, respectively, before pulmonary administration of the therapeutic protein or peptide, or remained untreated. The rats received 5, 50, or 500 μg of IgG, 100 μg (840 IU) of hCG, or 40 μg (465 mIU) of human insulin by intratracheal instillation (100 μl of 0.9% NaCl solution) or 5 or 50 μg of IgG, 10 μg of hCG, or 10 μg of human insulin by IV injection (500 μl of 0.9% NaCl solution).

The three doses of IgG delivered to the lungs were chosen according to the quantities of total IgG naturally present in the lungs of untreated rats (52 ± 13 μg, as measured in BAL). We hypothesized that a low (5 μg), an equivalent (50 μg), and a high (500 μg) dose comparative to local levels would allow us to observe the activation of increased IgG clearance once levels exceeded normality.

Serum was collected by orbital bleeding at intervals up to 42 days, 3 days, or 6 h after administration of IgG, hCG, or insulin, respectively. IgG concentrations in serum were measured by enzyme-linked immunosorbent assay. Plates (Nunc-Immuno Plate Maxisorp Surface, GIBCO-BRL Life Technologies) were coated with dinitrophenyl human albumin, incubated with dilutions of sera, and developed with horseradish peroxidase-conjugated anti-rat IgG Fc region. Serum levels of hCG and insulin were measured by enzyme immunoassay (Biosource, Nivelles, Belgium) and immunoradiometric assay (Biosource), respectively.

The areas under the serum concentration-time curves (AUC) were calculated using the linear trapezoidal rule, and the absolute bioavailability of intratracheal instillation was calculated by comparison to IV injection as

$$\frac{AUC_{IT} \cdot dose_{IV}}{AUC_{IV} \cdot dose_{IT}} \cdot 100 \quad (1)$$

Statistics. The data were validated by the Dixon test. Results are expressed as means ± SE. ANOVA and Tukey's test were performed to demonstrate statistical differences ($P < 0.05$) using the software Sigma-Stat for Windows (SPSS, San Rafael, CA).

RESULTS AND DISCUSSION

Fate of IgG in the lungs. To visually assess the fate of a large macromolecule in the lungs, we delivered rat IgG to the lungs of rats and examined protein fate in pulmonary tissue postdelivery using confocal laser scanning microscopy. FITC-labeled IgG slowly disappeared from the pulmonary tissue over a period of 24 h after intratracheal spray-instillation (Fig. 1, *A–C*). An intense uptake of IgG (that primarily appeared in soluble form, although some particulate IgG was also visible; see Fig. 1*B*) by AM arose as early as 15 min after delivery and remained prominently visible for up to 3 days (Fig. 1, *A–D*). This finding coincides with previously published observations of AM uptake for different-sized macromolecules (10, 19, 31).

Effect of AM uptake on systemic bioavailability. Whereas previous studies either did not assess (10, 19, 31) or concluded negligible (3) the effect of AM protein uptake on systemic bioavailability, we hypothesized that an important local elimination mechanism of macromolecules involved clearance by AM and hence that its inhibition could lead to significant

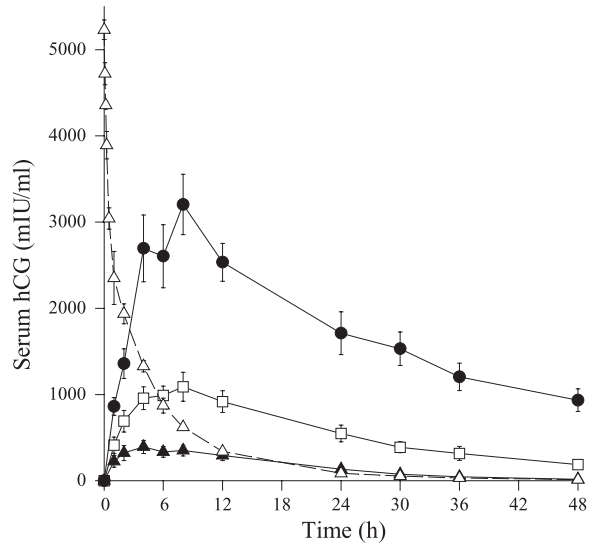

Fig. 6. Pharmacokinetics of pulmonary hCG in AM-depleted and control rats. Rats received a hCG dose of 100 μg by intratracheal instillation 1 day after Cl_2MDP (●, $n = 4$) or PBS (□, $n = 6$) liposome administration or no treatment (▲, $n = 6$). The bioavailability of hCG increased from 4.4 ± 1.1% (untreated rats) to 17.6 ± 3.7% (PBS liposomes) to 59.7 ± 9.2% (Cl_2MDP liposomes) compared with an intravenous dose of 10 μg (△, $n = 4$). Values are averages ± SE.

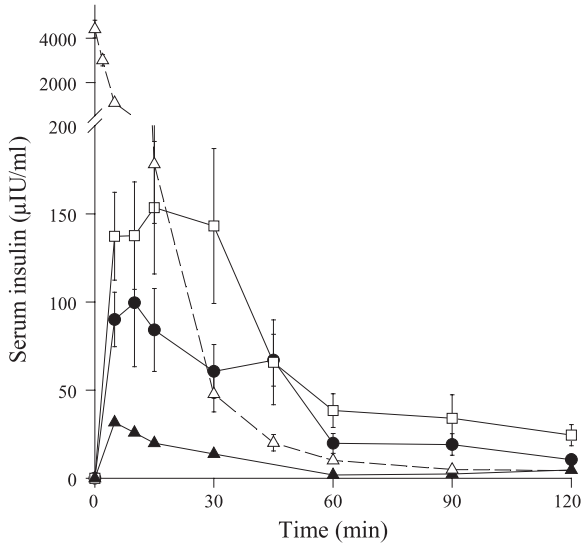

Fig. 7. Pharmacokinetics of pulmonary insulin in AM-depleted and control rats. Rats received an insulin dose of 40 μg by intratracheal instillation 1 day after Cl_2MDP (●, $n = 7$) or PBS (□, $n = 7$) liposome administration or no treatment (▲, $n = 6$). The bioavailability of insulin increased from 3.1 ± 0.7% (untreated rats) to 13.3 ± 3.4% (PBS liposomes) and 8.7 ± 2.9% (Cl_2MDP liposomes) compared with an intravenous dose of 10 μg (△, $n = 6$). Values are averages ± SE.

enhancement of pulmonary bioavailability. To verify this, we eliminated AM using liposomes containing Cl_2MDP and compared serum IgG levels after pulmonary administration of IgG to AM-depleted rats and control rats. Selective depletion of splenic, liver, or AM by Cl_2MDP liposomes has largely been used to study macrophage function in physiology, pathology, or immunity (2, 26). In our experiments, a single IT dose of Cl_2MDP liposomes in rats decreased AM number in BAL from 2.3 ± 0.4 million to 0.5 ± 0.1 million at *day 1* after delivery (Fig. 2*A*). AM number remained low for the next 36 h, and repopulation of the lungs with AM was observed at *day 5*. In contrast, animals treated with liposomes prepared with PBS and no Cl_2MDP showed normal AM population throughout the period examined (Fig. 2*A*). IT instillation of Cl_2MDP or PBS liposomes or of simply 0.9% NaCl caused a slight and similar neutrophil influx in the air spaces ($P > 0.10$; Fig. 2*B*) and no structural alterations to airway or alveolar epithelia (Fig. 3), as previously reported (2, 13). Regions of atelectasis were apparent in histological lung sections after administration of liposomes (Fig. 3, *B* and *C*). One day after liposome treatment, rats were intratracheally instilled with 500 μg of IgG, and the subsequent absorption of IgG in blood that occurred is shown in Fig. 4. A substantial rise in serum IgG levels resulted from the depletion of AM; the pulmonary bioavailability relative to IV injection increased from 4.7% in untreated rats (or 4.5% in PBS liposome-treated rats) to 10.5% in Cl_2MDP liposome-treated rats (Fig. 4; $P < 0.05$). Conversely, when we instilled additional AM into rat lungs, we observed a decrease in pulmonary bioavailability of IgG to 2.6% (Fig. 4; $P < 0.05$). In all cases, serum IgG levels were affected by AM number within the first hours of the absorption phase (Fig. 4), indicating that AM clearance was rapidly effective. The responsiveness of IgG absorption from the lungs on AM number demonstrates that AM present a significant hindrance to IgG transport from airway lumen to the bloodstream, contrary to previous arguments for a lesser role (8, 10, 25, 32).

Application to other macromolecules. To assess the significance of AM at limiting the systemic absorption of macromolecules from the lungs in general, we visualized the fate of hCG (39.5 kDa) in alveoli and studied its transport from air spaces into blood in AM-depleted rats and control rats. hCG was selected both for its relatively lower-molecular-weight and because AM have no receptors for hCG (24), which is not the case for IgG, recognizing nonetheless that Fc receptors on phagocytes have high avidity for antibodies bound to antigen and relatively low affinity for free immunoglobulin molecules (11). FITC-hCG slowly disappeared from the lung tissue and was intensely taken up by AM, similarly as FITC-IgG (Fig. 5*A*). The serum hCG levels that followed the pulmonary administration of a solution of hCG were increased 3 times in rats treated with PBS liposomes and 8 times in rats treated with Cl_2MDP liposomes compared with rats whose lungs remained untreated (Fig. 6; $P < 0.05$). This corresponded to an enhancement in absolute bioavailability of pulmonary hCG from 4.4% (untreated rats) to 17.6% (PBS liposome-treated rats; $P < 0.05$) to 59.7% (Cl_2MDP liposome-treated rats; $P < 0.05$) and therefore to an enhancement in hCG transport due to AM depletion that was larger than that observed for IgG (Fig. 4). This indicates that AM were a greater barrier to hCG than IgG absorption and hence that other mechanisms, e.g., mucociliary

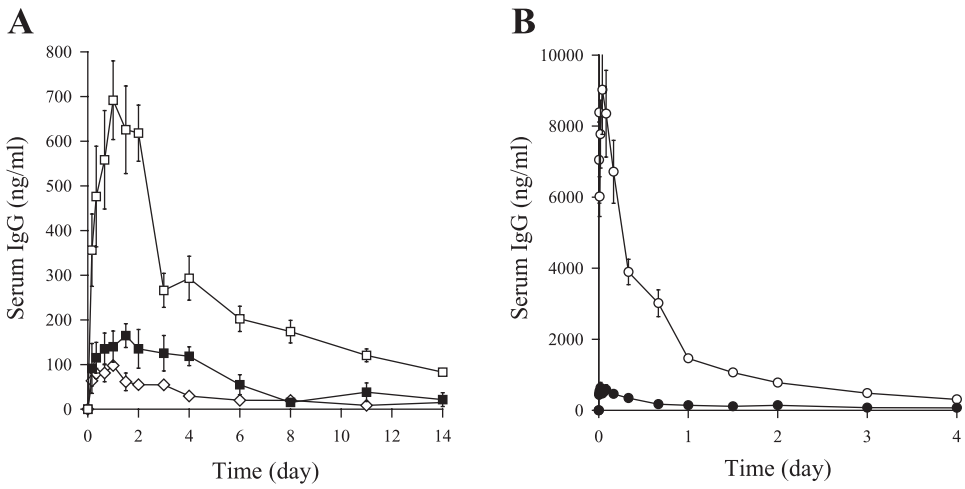

Fig. 8. Pharmacokinetics of pulmonary and intravenous IgG at variable doses. Rats received a dose of 500 μg (□, $n = 6$), 50 μg (■, $n = 3$), or 5 μg (◇, $n = 3$) by intratracheal instillation (*A*), or a dose of 50 μg (○, $n = 4$) or 5 μg (●, $n = 4$) by intravenous injection (*B*). The 500-, 50-, and 5-μg doses instilled corresponded to an absolute bioavailability of 4.7 ± 1.1%, 10.9 ± 2.6%, and 38.4 ± 9.6%, respectively. Values are averages ± SE.

clearance, might have taken over in the elimination of IgG from the lungs. The influence of AM on both hCG and IgG transport suggests that AM uptake predominantly involves adsorptive and/or fluid-phase endocytosis instead of receptor-mediated endocytosis (23–24). Similarly to IgG, the depletion of AM resulted in increased absorption of hCG into blood early over the absorption phase (within 1 h, Fig. 6).

The uptake of fluid by endocytosis is a natural characteristic of professional phagocytes (22). Because uptake is low but uninterrupted, significant internalization of tracers needs periods of several minutes to hours (17). Given these features, we expected AM clearance to be a most significant barrier to pulmonary absorption of macromolecules with hours of persistence in the airway lumen and to have no impact on pulmonary absorption of peptides, which are cleared within minutes.

To verify this assumption, we assessed the impact of AM on pulmonary absorption of the peptide insulin. Confocal imaging showed that FITC-insulin was eliminated rapidly from the lungs, with an apparent binding to tissue fibers but no uptake by AM (Fig. 5, *B* and *C*). Transport of instilled insulin to the bloodstream increased after lung treatment with PBS liposomes ($P < 0.05$) but, in contrast to IgG and hCG, no additional increase was associated with AM depletion due to Cl_2MDP (Fig. 7; $P > 0.10$). The absolute bioavailability of pulmonary insulin was 3.1, 13.3, and 8.7% in untreated, PBS liposomes and Cl_2MDP liposome-treated rats, respectively ($P < 0.05$, PBS and Cl_2MDP liposomes vs. untreated rats; $P > 0.10$, PBS vs. Cl_2MDP liposomes). This shows that Cl_2MDP liposomes did not cause additional permeability change to the epithelium compared with PBS liposomes and that AM clearance is a slow process that does not impact on pulmonary absorption of peptides.

Strategies for enhancing pulmonary bioavailability. Our studies suggest that pinocytic uptake by AM represents a degradation pathway for inhaled proteins, which competes with absorption of proteins across lung epithelia and thereby lowers pulmonary bioavailability to the degree that the rate of AM uptake and degradation is near to or greater than the rate of transport from the lung lumen into the bloodstream. On the basis of this conclusion, we hypothesized two strategies to increase pulmonary bioavailability. A first conventional strategy is suggested by the enhancement in pulmonary absorption of hCG and insulin that followed pretreatment of the lungs with PBS liposomes (Figs. 6 and 7) and consists of increasing the rate of protein transport across the alveolar epithelium by chemical means (14, 15, 18). In this regard, the coadministration of peptide drugs and of dipalmitoylphosphatidylcholine, the most abundant phospholipid component of pulmonary surfactant, has been reported, interestingly, to accelerate and increase drug absorption from the lungs in rats, whatever the mode of delivery, instillation of a physical dispersion in saline or inhalation of a dry powder aerosol (6, 21).

A second strategy, implied by our experiments involving Cl_2MDP liposomes, requires lowering the rate of AM uptake to increase pulmonary bioavailability. For proteins such as IgG, for which primary cultured rat epithelial cell monolayer studies have shown protein absorption to be a saturable process (12, 20) [pinocytosis by macrophages is in contrast a sustained phenomenon (22)], decreasing the doses of the protein delivered to the lungs might favor absorption to the systemic circulation relative to local degradation. To assess this hypothesis, we delivered to rat lungs IgG doses of 500, 50, and 5 μg and observed less than a proportional decrease in serum IgG levels (Fig. 8*A*), whereas decreasing the IgG dose injected intravenously from 50 to 5 μg did cause a proportional decrease in serum IgG levels (Fig. 8*B*). This translates into an increase in absolute IgG bioavailability from 4.7 to 10.9 ($P < 0.05$) to 38.4% ($P < 0.05$) for the 500-, 50-, and 5-μg doses instilled, respectively. The absolute bioavailability of 1.5% previously reported in rats at a dose of 5 mg is consistent with this trend (9).

Chemical or physical alternatives of minimizing AM clearance relative to absorption include the inhibition of endocytosis using physiological modulators (4, 17), the coadministration of ligands competing with proteins for binding on macrophage plasma membranes (17), and the preparation of large porous particles that could protect the drug from local pinocytic and phagocytic degradation and release it at a rate slightly slower than the rate of absorption (7). These approaches could be appropriately applied to macromolecules due to their long residence times in the alveoli, but could also be extended to sustained-release formulations of molecules that lack physical protection against AM clearance. Systemic drug absorption after inhalation could be improved, but therapeutics or prophylactic drugs delivered to the lungs for a local action could improve in efficacy from these strategies as well.

ACKNOWLEDGMENTS

We thank Lipoid for donating lecithin, the Cell Biology Unit [Université Catholique de Louvain (UCL), Brussels, Belgium] for the use of the confocal microscope, and the Industrial Toxicology and Occupational Medicine Unit (UCL, Brussels, Belgium) for help with cell counting and differentiation in BALs.

R. Vanbever is a Chercheur Qualifié of the Fonds National de la Recherche Scientifique (Brussels, Belgium).

GRANTS

This work was funded in part by Advanced Inhalation Research (Alkermes; Cambridge, MA).

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