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Mechanisms that determine nanocarrier targeting to healthy versus inflamed lung regions

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Abstract

Inflamed organs display marked *spatial heterogeneity* of inflammation, with patches of inflamed tissue adjacent to healthy tissue. To investigate how nanocarriers (NCs) distribute between such patches, we created a mouse model that recapitulates the spatial heterogeneity of the inflammatory lung disease ARDS. NCs targeting the epitope PECAM strongly accumulated in the lungs, but were shunted away from inflamed lung regions due to hypoxic vasoconstriction (HVC). In contrast, ICAM-targeted NCs, which had lower *whole-lung* uptake than PECAM/NCs in inflamed lungs, displayed markedly higher NC levels in inflamed *regions* than PECAM/NCs, due to increased regional ICAM. Regional HVC, epitope expression, and capillary leak were sufficient to predict intra-organ of distribution of NCs, antibodies, and drugs. Importantly, these effects were not observable with traditional spatially-uniform models of ARDS, nor when examining only whole-organ uptake. This study underscores how examining NCs' *intra-organ* distribution in spatially heterogeneous animal models can guide rational NC design.

Key words: Spatial heterogeneity; Patchy; Inflammation; ARDS; Nanoparticles; Nanocarriers; Nanoparticle biological interactions; Whole organ distribution; Nano-bio interface

Nanomedicine has made great progress in targeting nanocarriers (NCs) to individual organs.[1–5] However, within an organ, diseases display a large degree of *spatial heterogeneity*, with the same organ containing both healthy and pathological regions.[6–10] Thus, some NCs may appear to efficiently target an organ, but it remains unknown if those NCs in fact target the pathological subregions rather than the nearby healthy regions. To address this question, a major need exists for animal models which recreate the spatial heterogeneity seen in human diseases.

Therefore, we created a new mouse model to study how NCs distribute within a diseased organ that displays the spatial heterogeneity typical of human diseases. We chose to focus on inflammatory disorders, since they constitute a very large class of diseases, change organ physiology significantly, and nearly all

Abbreviations: PATH ratio, Pathologically Altered To Healthy tissue ratio; NC, nanocarrier; PECAM, platelet endothelial cell adhesion molecule-1; ICAM, intercellular cell adhesion molecule-1; ARDS, acute respiratory distress syndrome.

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display spatial heterogeneity in their severity. We chose as our model inflammatory disease ARDS (acute respiratory distress syndrome), which is an acute, diffuse, inflammatory lung injury that kills ~75,000 Americans annually.[11] In ARDS, the capillaries of the lungs' alveoli (air sacs) increase their permeability, causing the alveoli to fill with edema liquid and neutrophils,[12] similar to the leukocyte-rich tissue edema present in nearly all inflammatory diseases. Notably, in every ARDS patient, these inflammatory changes are only found in scattered patches of the lung,[13,14] making ARDS an archetype of spatially heterogeneous organ inflammation. Targeting these inflamed lung regions has been the goal of numerous labs' NCs and a program to develop liposomal drug delivery for ARDS,[15–19] but these NC studies only examined whole lung uptake, and not whether the NCs actually reached the inflamed lung regions.

For the present study, we initially focused on the most-studied of these NCs: NCs coated with anti-PECAM antibodies (PECAM/NCs), which have shown very high whole lung uptake in ARDS models in mice, rats, and pigs.[18,20,21] In our mouse model of spatially heterogeneous ARDS, we found that PECAM/NCs accumulate preferentially in healthy lung regions, not the intended inflamed lung regions, due to hypoxic vasoconstriction. Surprisingly, however, we found that other NCs and pharmacological agents actually preferentially accumulated in the inflamed lung regions. Experimental and computational studies showed that these diverse distributions were determined by 3 simple transport mechanisms, which allowed us to design a new targeted NC, ICAM/NC, that shows strong preference for the most inflamed regions. Notably, ICAM/NCs appear inferior to PECAM/NCs in traditional, spatially-uniform ARDS models, with lower whole-lung uptake than PECAM/NCs even in inflamed lungs. Only by comparing ICAM/NCs vs. PECAM/NCs in a spatially heterogeneous animal model and examining intra-organ distribution did it become clear that ICAM/NCs achieve higher *local* concentrations in the inflamed areas in need of pharmacotherapy. These findings highlight the importance of developing spatially heterogeneous animal models like the one introduced here.

Methods

Unilateral LPS instillation

C57BL/6 adult mice were instilled with LPS (1 mg/kg). For the traditional "diffuse LPS" model of ARDS, the LPS was instilled via insertion of a 29-gauge tuberculin syringe into the trachea. For unilateral LPS, the mice were anesthetized with ketamine and xylazine followed by endotracheal intubation with a 20-gauge angiocatheter. A PE-10 catheter (outer diameter 0.024″) was inserted and positioned so that it terminated within the superior lobe, and the LPS was instilled as a 1 μL/kg solution. Twenty-four hours later, assays of lung distribution (NC injection followed by sacrifice 30 min later) and lung inflammation were performed as previously described.[22]

Nanoparticle production

Liposomes were made by first creating lipid films in round-bottom glass vials: 1×10^{-5} mol of lipids in chloroform were added per vial, followed by chloroform evaporation via nitrogen stream and then at least 2 h of lyophilization. Lipids (from Avanti) were mixed at molar percent: DPPC 52%, cholesterol 45%, DSPE–PEG-2000–maleimide 2%, with the remaining 1% being either DSPE–PEG-2000–DTPA (for In^{111} labeling experiments), PE–lissamine–rhodamine (for fluorescent tracing), or DPPC (for all other experiments). Lipid films then had 0.5 mL PBS added, warmed to 50°C, bath sonicated at 50°C for 10 s, followed by extrusion (Avanti syringe extruder) through a membrane with 200-μm pores, producing ~2×10^{13} liposomes/mL.[18]

Antibodies were conjugated to maleimide-liposomes by SATA–maleimide conjugation chemistry. Briefly, a 6× excess of SATA (Sigma) was added to antibodies at room temperature (RT) for 30 min, generating 1 sulfhydryl group per IgG molecule. The acetylated sulfhydryl of the SATA moiety by adding hydroxylamine (50 mM final concentration) and incubating for 2 h at RT. Then maleimide-containing liposomes were mixed with the deprotected SATA-antibodies to generate liposomes that bore approximately 200 antibodies per liposome.[18] Unconjugated antibodies were removed by centrifugation at 32,000×*g* for 1 h to pellet the conjugated liposomes, and discarding the supernatant containing free antibodies.

The liposomes were radiolabeled with I^{125} as described previously[18]: 10% of their coating antibodies be I^{125}-labeled IgG. I^{125}-labeling of IgG utilized Pierce iodination beads, conducted after SATA-conjugation to the antibodies.

For digital autoradiography (DAR), liposomes containing 1% DSPE–PEG-2000–DTPA were labeled with In^{111}: In^{111} source (150 μCi; from Nuclear Diagnostics Products) was mixed with 2 M trimethyl acetic acid (pH 4.5) and pH set to 4.5, and then mixed at with antibody-conjugated liposomes at a volume ratio of 2:1 liposomes:In^{111}-TMAA, incubated at room temperature for 1 h, and then centrifuged at 32,000×*g* for 1 h. In^{111} loading showed the following: 91% of the In^{111} was found in the pellet, and on thin layer chromatography (TLC) in 2 different mobile phases (9% NaCl, 10 mM NaOH, and 10 mM EDTA), 99% of the In^{111} migrated with the liposomes in both mobile phases.

Nanogels (lysozyme–dextran nanogels of 300 nm) were produced via the method previously described.[23]

Nanoparticle tracing in vivo

For multi-organ biodistribution, mice were injected with 2×10^{11} liposomes/mouse, which is equivalent to 5 mg/kg of total lipid. Nanogel injections were similarly ~1×10^{11} nanoparticles per mouse.

Digital autoradiography (DAR) was performed by injecting the mice with In^{111}-labeled liposomes. These liposomes contained 1% sacrifice 30 min later, and preparation of fresh-frozen lungs in OCT. Lung blocks were cut on a cryostat microtome, with the slides then developed on a phosphor screen plate overnight followed by imaging the screen on a Typhoon FL7000.

Fluorescent liposomes were injected and the lungs prepared identically for those of In^{111}-labeled liposomes. Fluorescent images were taken on an Aperio slide scanner.

Tc99m-MAA (macroaggregated albumin) was purchased from Nuclear Diagnostics Products, and injected into mice in a