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COMMENTARY

DRUG TARGETING TO THE LUNGS

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Drug targeting is on the verge of a rapid expansion that will antiquate many of today's delivery methods by the year 2000. Pulmonary targeting is at the forefront of this development because access by both intravenous and transtracheal routes is direct and relatively noninvasive. Scientific, pharmaceutical, economic, and clinical factors are combining to accelerate these developments. The most important factor is clinical need. Our remarkable success in treating diseases with large host-invader differences has left us with serious remanent diseases in which the biological differences are small or exotic, drugs of choice toxic, and host defenses compromised. These diseases include neoplasia; deep fungal, parasitic, and viral infections; autoimmune disorders; genetic diseases; and sequellae of aging. Although new molecular entities with improved specifity will continue to become available at a rate of two to three per year, many of the drugs we need for even the most refractory diseases are available now and would be highly effective if protected against inactivation, targeted appropriately, and released at controlled rates. Physicians have gradually eased constraints on patient administration of parenteral drugs. An example is the inhalant form of bronchodilators. Major pharmaceutical houses have become aware that parenteral formulations (a necessity for high-efficiency targeting) can provide the marginal profits needed to justify their development. Also, federal examiners are increasingly experienced at evaluating drug–device combinations. Until recently, targeting devices were the sole prerogative of oncology review groups. Now they are starting to be evaluated by examiners of antibiotic agents. A better appreciation of differences in the targeting efficiencies of various devices appears to be leading towards more appropriate regulations for each device. The most recent factor contributing to growth of this field is the new scientific information on: (a) endothelial receptors that enhance pulmonary drug clearance, (b) the effects of disease on endothelial and sequestered tissue receptors, (c) receptor-binding substances that can be used to make targetable drug carriers, (d) technologies for improving the formulation of carriers and coatings, (e) ways to improve the mass-production of complex biological molecules (biopharmaceuticals) needed for drug targeting, and (f) *noninvasive* means for monitoring free drug levels and drug clearance rates within tissues (principally by nuclear isotopic and nuclear magnetic resonance methods). For comprehensive reviews of targeting and sustained release, the reader is referred to the following sources [1–4]. The major classifications of pulmonary targeting are summarized in Table 1. This commentary will emphasize emerging biophysical and biochemical approaches to pulmonary targeting by the vascular route.

General considerations. Vascular targeting to lung parenchymal cells involves two major steps because of the existence of an anatomic and functional endothelial barrier. *First-order targeting* involves the clearance of an agent by the lung. This includes capture in the desired capillary bed and passage across the endothelium into the tissue compartment. *Second-order targeting* involves the subsequent capacity (if any) of an agent to select specific cell types within the tissue compartment (e.g. tumor versus normal). It would be desirable to accomplish both steps with affinity agents alone, such as monoclonal antibodies. However, first-order targeting is largely obstructed by the vascular barrier [1, 2, 4]. Pulmonary diseases, carcinomas in particular [5], can lower this barrier. However, it usually remains sufficiently intact to prevent circulating drug carriers

Table 1. Pulmonary drug targeting

I. Method
  A. Biochemical
    1. Enzyme binding (intravascular thrombi)
    2. Receptor binding (hormones and specific drugs)
    3. Antibody binding
      a. To target structures
      b. To targeting agents (platelets, WBC, RBC)
  B. Biophysical
    1. Embolic trapping (size)
    2. Magnetic capture
    3. Altered microvascular barriers
    4. Regional hyperthermia with temperature-sensitive liposomes
    5. Regional perfusion
    6. Inhalation
  C. Bioadhesion—combination of biophysical and biochemical (for particles and capsules)
II. Carrier
  A. Small molecules
  B. Macromolecules
  C. Supramolecular carriers
    1. Microspheres and nanospheres
    2. Liposomes
    3. Microemulsions
    4. Hollow vesicles
    5. Multi-stage devices (see text and Fig. 1)
    6. Cells (intact, enucleated, inflammatory, tumor)

of > 45,000 molecular weight from localizing primarily in the lungs. Instead, they are cleared by the liver, spleen and bone marrow [2, 4], and to a lesser extent by the kidneys. Similar biodistributions are observed for most diagnostic agents, but the consequences are generally ignored because toxicities are usually low and only a small percentage of the agent must localize in lesional tissue in order to detect it.

In assessing drug localization, the accepted pharmaceutical criterion for targeting is that the therapeutic index of a drug must be increased by at least one-half an order of magnitude. Hence, a true targeting method should cause liver, bone-marrow and kidney levels to rise by less than one-third of the pulmonary increment. There are two exceptions to this. The first is site-avoidance targeting, in which a drug is allowed to concentrate in multiple nontarget organs as well as lung, provided it avoids its major organ(s) of toxicity. One example is adriamycin, an antitumor agent whose predominant toxicity is dose-dependent, irreversible cardiomyopathy [6]. The second exception occurs when a nontoxic drug is localized for reasons of rapid intravascular degradation, high production costs or limited availability. In choosing an optimal route of drug administration, it is important to define the purpose of therapy. For prophylaxis against infection or tumor metastasis, as well as modulation of physiologic pulmonary functions (e.g. bronchodilitation), the inhalation route gives acceptable drug access. However, for treatment of established infections and tumors, the accompanying physical barriers of obstruction and atalectasis almost always impair access of inhalant drugs to the most severe foci of diseases. For example, in invasive pulmonary aspergillosis, where amphotericin B has been given by inhalation to reduce systemic toxicity, no major therapeutic advantages have been reported [7]. Although physiologic alterations in ventillation–perfusion ratios also decrease blood flow through the nonaerated foci, vascular access is still superior from the standpoint of clinical response. In selecting a targeting device, one must also determine if the purpose of therapy is to modulate pulmonary physiology (for which the preferred agent is a continuously circulating drug with affinity for lung endothelium), or to treat established obstructive infections or tumors (for which the preferred agent is a sustained-release drug device targeted to the lesional stroma and, if possible, caused to adhere to lesional cells).

Magnetic targeting. In malignancies and life-threatening infections, it is desirable to target drugs with very high efficiency. In the following example, such targeting was required in order to fulfill the dual objectives of modulating local host resistance and minimizing systemic toxicity of a circulating biomodulator that would be lethal if given in a nonencapsulated form. This example is discussed in relative detail because magnetic carriers have produced the greatest increments (multiples of 10–100) in therapeutic index of any targetable depot-type device [4, 8], and they have reached an advanced stage of experimental development. Pulmonary targeting of the neutrophil chemoattractant, formyl-methionyl-leucyl-phenylalanine (fMLP), has been carried out in rats by entrapping the agent in magnetically responsive, 0.6 μm albumin microspheres [9] (Fig. 1). Magnetic responsiveness is conferred by a co-entrapped ferromagnetic material, Fe_3O_4 (20%, w/w). Because the spheres are considerably smaller than the 4 μm size which separates nonembolizing from embolizing particles, they would normally pass through the target vessels and be cleared by the liver and spleen. Instead, they are captured in the small arterioles and capillaries of the lungs with an external gradient magnet of intermediate field strength (0.55 Tesla). Approximately 40% of the spheres localize in thoracic viscera (35% in lungs and 5% in heart). Magnetic capture takes place within seconds of injection. The vascular level of localization is based on a competition between flow and magnetic forces. At linear flow rates ≤ 0.75 cm/sec, the magnetic force exceeds that of blood flow, and the spheres are dragged up the magnetic gradient and caused to pass between and through endothelial cells into the interstitium [4, 9]. Extravascular migration is

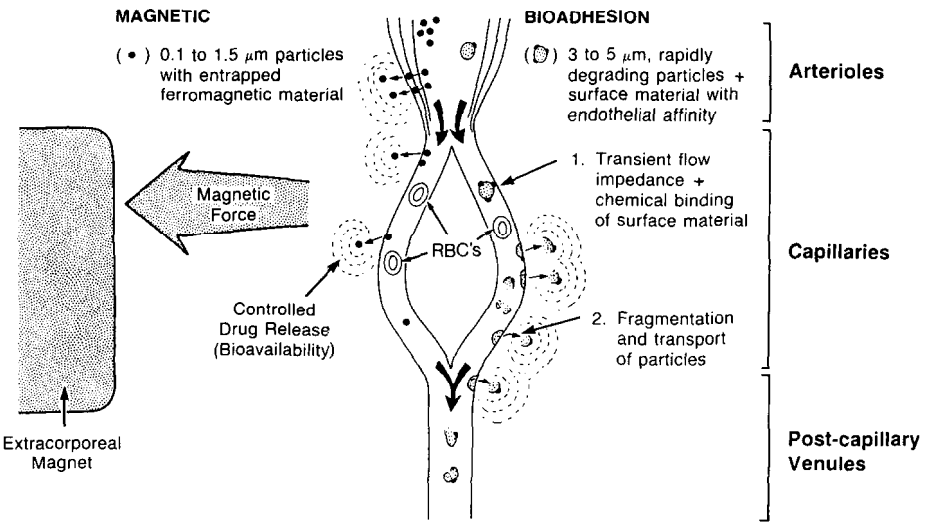

Fig. 1. Methods of targeting.

complete within 5–10 min. Due to the extreme rapidity of targeting and the relatively delayed release of drug ($T_{\frac{1}{2}} = 20$ min) from a particulate class of carrier, almost all the fMLP is deposited in either the lungs (25%) and heart (3%), or in the reticuloendothelial organs (liver = 49%; spleen = 8%) and kidneys (11%). Because of this high efficiency of pulmonary targeting, the low total dose of fMLP (15.7 μg/kg), and the rapid reticuloendothelial clearance of untargeted drug, circulating levels of *free* fMLP remain very low (1.9×10^{-8} M). Within target tissues, the controlled release of fMLP in conjunction with its lipophilic nature (which facilitates membrane binding) combine to maintain a tissue reservoir of the entrapped + released agent. Hence, total tissue fMLP stays nearly constant between 5 min (2.9×10^{-6} M) and 70 min (2.7×10^{-6} M). Local drug release sets up chemical gradients around each collection of microspheres. This broadcasts an attractant signal to circulating neutrophils which migrate out into the interstitium. When the fMLP dose is selected to give tissue levels above *ca.* 3.3×10^{-8} M, these neutrophils are further induced to release O_2^- and lysosomal enzymes [10]. This produces localized, acute tissue injury and initiates subsequent inflammatory processes. The targeted fMLP also attracts resident alveolar macrophages [9]. These cells engulf almost all the microsphere material that has passed across the lung septa into the small airspaces. The 25% pulmonary localization of total injected drug which is obtained in this study is markedly higher than the 0.1–0.5% commonly obtained with standard drugs and the 2–5% observed for monoclonal antibody–drug conjugates [11]. Optimization of magnetic field configurations and use of the newer, high susceptibility magnetic materials [12] are expected to double this targeting efficiency. The LD_{20} of nonembolizing albumin-Fe_3O_4 spheres is 400 mg/kg. To put this in perspective, animal studies of tumor therapy with adriamycin-Fe_3O_4 spheres routinely employ carrier doses of 1/80th to 1/16th the LD_{20} dose, depending on the volume of target tissue [13]. Clearance of remanent albumin from the lungs occurs within 5–15 hr. Secondary accumulation occurs in the liver and spleen, and excretion takes several days. By contrast, a significant percentage (20–45%) of targeted Fe_3O_4 stays in the lungs for up to several months. The remainder is processed by monocyte-macrophages and is both relocated to the liver (majority) and excreted by the kidneys. The toxicity of remanent pulmonary Fe_3O_4 is low. A review of the world literature indicates that miners who inhale Fe_3O_4, and live for decades with lung concentrations 100 times those deposited by magnetic targeting, exhibit no increased incidence of pulmonary fibrosis or primary lung cancer [14]. Nevertheless, the lack of complete biodegradability has influenced federal regulations on ferromagnetic drug-matrix devices and has limited their anticipated applications to life-threatening disease. Another problem with their generalized use is the technical expertise, specially designed magnets, and magnetic monitoring devices needed to obtain homogeneous targeting throughout tissue volumes as large as the human thorax. Such homogeneity is necessary to prevent focal overtreatment and tissue necrosis by highly toxic drugs. This will likely restrict pulmonary targeting to specialized centers. It still remains to be demonstrated that homogeneous magnetic capture can be achieved over thoracic volumes. A realistic, although relatively expensive, solution is offered by an electromagnet of 2-Tesla field strength and quadripolar configuration which maintains a field *gradient* of constant magnitude over a 35–40 cm pole gap. Two other potential problems exist for the first-generation carriers: (a) although the stabilized albumin matrix gives prolonged drug release *in vitro* [15], its rapid biological clearance *in vivo* limits drug coverage to approximately 8 hr; (b) unlike the case for lipid-soluble fMLP (above), small, water-soluble drugs, upon release, will diffuse much more freely within the tissues. In the process, they re-enter adjacent capillaries and are cleared from the lung. Accelerated clearance partially abrogates the benefit of targeting. The latter two problems are potentially resolved as follows: (a) albumin microspheres can be replaced with a more inert, biocompatible matrix made of poly-L-lactic acid [1, 3] or poly-glutamic acid polymers [3], which give extended tissue retention times and local drug release intervals of several days to a year; (b) water-soluble drugs can be reformulated as polymeric conjugates which, upon release, are retained much longer in the tissue compartment. It is also important to note that tissue microspheres undergo significant uptake by both tumor cells and intralesional macrophages. Hence, the related fraction of water-soluble drug is released intracellularly and is not available for diffusional clearance.

Magnetic albumin-adriamycin spheres are in an advanced phase of animal testing [13]. These results will be important for subsequent formulations of interest to pulmonary medicine. For example, amphotericin B microspheres could prove useful for treating pulmonary aspergillosis in the presence of severe renal toxicity [16], and for potentiating the effects of antitumor drugs in the treatment of lung cancer [17]. Spheres of fMLP may prove useful as an adjuvant approach to the treatment of pulmonary aspergillosis [9], because the resistant hyphal forms which prevail in established infections are susceptible primarily to extracellular killing by neutrophil products [18]. The C-methyl ester of fLMP peptide (fMLP-M) has 1000 times the affinity for monocyte-macrophages as does fMLP [19]. Microspheres of this peptide might be effective as an adjuvant formulation for treating pulmonary neoplasms. Urokinase has been successfully entrapped and released from magnetic spheres *in vitro* [4]. If effective *in vivo*, such spheres might reduce toxicity during fibrinolytic enzyme therapy of pulmonary emboli and coronary arterial thrombi. Also, magnetically targeted mediators have been used recently to study basic disease processes in ways that were not possible before. For example, targeted fMLP induces neutrophil inflammation which is more effectively localized in the lung than the inflammation resulting from nontargeted initiators, such as cobra vernum factor [20] and zymosan-activated serum [21]. Hence, mediator targeting provides a rapid *in vivo* assay for screening pharmaceutical agents that modulate neutrophil migration or neutrophil-mediated inflammation. It might be used, for example, to test

the capacity of a new, orally administered agent to block the degradation of lung elastin by neutrophil elastase.

Targeting by embolic trapping. Recently, 40 μm biodegradable starch microspheres (commercially available as Spherex) have been used in animals for the selective occlusion of hepatic arterial blood flow to tumors [22]. Similar spheres are just starting to be tested in humans. In animals, they retard tumor growth significantly, but the effect is only temporary. Host survival is extended when embolization is combined with local hyperthermia. Similar approaches might be useful for pulmonary neoplasms, because the lungs, like the liver, have a double blood supply (bronchial + pulmonary). At the usual stage of clinical detection, most pulmonary neoplasms (including the centrally located squamous-cell tumors, peripheral adenocarcinomas, and metastatic tumors) receive a large fraction of their blood supply from bronchial arteries (or chest-wall collaterals). These arteries are accessible to selective catheterization, and such procedures are performed not infrequently for control of arterial bleeding from tumors and sclerosis of vascular malformations [23]. The starch microspheres described above are designed to degrade within 25–30 min of embolic localization. This degradation is mediated by blood amylase. The result is that these spheres produce only transient vascular occlusion. However, occlusion intervals can be extended by repeated injection. It has been proposed that the property of rapid degradation offers the flexibility of subsequent regional perfusion of the embolized arterial tree with chemotherapeutic agents [22]. Although such regimens have yet to be tested, an extensive radiological literature [23] indicates that the permanent sclerosing agents, such as isobutyl-cyanoacrylate, are more effective in preventing subsequent outgrowth of blood collaterals that are often inaccessible to catherization. These permanent-type agents produce a continuous "cast-like" polymeric coating which adheres to the vascular endothelium. This results in more complete occlusion that can be achieved with either biodegradable or nonbiodegradable microspheres. Nevertheless, degradable starch spheres represent an excellent prototype device for designing nonmagnetic, multi-stage, bioadhesion devices (Fig. 1). The ideal formulation would be a 3–5 μm biodegradable sphere which would: give capillary-level distribution [24], control-release the appropriate drug [25], and have a surface coating that adheres to endothelium and promotes transport of drug-matrix fragments across the endothelial cells, as the intraluminal sphere disintegrates. The size uniformity required for reproducible degradation of these particles is readily achieved by micropore filtration.

Targeting due to altered microvascular barriers. Lesional accumulation of intravascular hematoporphyrins occurs spontaneously in squamous cell carcinomas of the lung [5, 26, 27]. The hematoporphyrin derivative, HPD (commercially available as Photofrin), and one of its fractions (Photofrin II) have been tested clinically for tumor-localizing and sensitizing properties. After allowing a sufficient interval for lesional accumulation, photoactivation is carried out bronchoscopically by laser excitation of the most brightly fluorescing mucosa. Tumor-cell killing results, in part, from intracellular generation of singlet oxygen. The largest absolute increments in tumor hematoporphyrin concentration have been achieved with a new fraction of HPD which has low polarity and exists in a highly aggregated state [27]. The mechanism of first-order uptake into tumor stroma is based in part on naturally occurring disruption of the microvascular barrier. Uptake is not restricted to carcinomas but also occurs in moderate and marked dysplasias [26]. Localization of unfractionated HPD in carcinomas is no more specific than that of ^3H_2O. Hence, HPD is not a true targeting agent. By contrast, the hydrophobic fraction of HPD localizes 2.5 times better than ^3H_2O and nearly as well as ^{67}Ga [27]. Unfortunately, because of aggregation, this fraction is concentrated in liver to a greater extent than in tumor. The failure of HPD and its derivatives to target is illustrated graphically by the skin photosensitivity which results if patients are exposed to sunlight within several weeks of treatment. Nevertheless, because the second step of HPD regimens involves photoactivation by bronchoscopic plus visual targeting of the laser, a localized therapeutic effect results. This emphasizes the general concept that two (or more) independent but anatomically overlapping interventions can produce a targeted effect even if only one of them is targeted, provided the nontargeted step is nontoxic. In this regard, several of the lasers used for photoactivation give off sufficient energy to produce mild local hyperthermia [5]. Hence, the HPD effect may actually result from two, coincidently targeted biophysical steps superimposed on relatively nontargeted drug accumulation.

The occurrence of naturally altered vascular barriers suggests the possibility of intentionally disrupting them with regionally perfused biomodulators. Candidates include: (1) hypertonic mannitol and dimethyl sulfoxide, which have been used clinically for transient disruption of the blood–brain barrier in experimental treatment of human tumors [28]; and (2) histamine and fibrinogen fragment D, which cause retraction and separation of endothelial cells [29]. From the mannitol studies, it can be concluded that barrier disruption, followed by regional perfusion of *free* drug, generally does not improve the therapeutic results. This is because disruption of the barrier exposes normal tissues to high acute concentrations of the drugs. The result is a marked increase in toxicity of most antitumor agents [28]. To improve the local therapeutic index, it will probably be necessary to perfuse targetable, *depot* devices, which give controlled release of the drug into lesional stroma.

Biochemical targeting. One of the most promising new approaches to first-order targeting is chemical binding to specific cell receptors in the lung. The *endothelial cell* is the key to clearance of intravenously administered drugs. Active uptake by the endothelium accounts for plasma removal of several biopharmaceuticals, including the two extensively studied biogenic amines, 5-hydroxytryptamine (5-HT) and norepinephrine (NE) [30]. In anesthetized dogs at moderate flow rates, the percentage pulmonary extraction of 5-HT and NE (injected into

the pulmonary artery at nanomolar doses) typically reaches 70 and 50% respectively [31]. It is possible, but untested, that drugs conjugated to these amines will undergo efficient pulmonary targeting. Because the endothelium of the limbs also actively clears these substances [31], any such conjugates would have to be (a) injected in doses sufficient to saturate the afferent vessels, (b) formulated as rapidly dissolving microparticles to minimize uptake during transit, or (c) injected through catheters in the right heart or pulmonary arteries. A second disadvantage of this approach is that 5-HT and NE exhibit significant pharmacologic activities of their own [32]. One potential solution involves synthesizing metabolically inactive derivatives which can still bind the transport site. A second is the conjugation of drugs to insulin, which is also actively cleared [33] and has a lower potential for acute pharmacologic effects. Antibodies to insulin receptors might also prove effective [34]. The prostaglandins (E and F) offer a third potential solution [32, 35], but these compounds are too unstable chemically to be ideal drug carriers. Alternatively, it may be possible to obtain pulmonary clearance with a group of natural products that binds to hydrolytic enzymes on the luminal surface of lung endothelium. These enzymes include 5′-nucleotidase [36] and angiotensin converting enzyme (ACE) [37]. They do not normally clear substrates but, instead, discharge the hydrolyzed products into the efferent circulation [37–39]. Although it remains untested, it may be possible to alter this fate by synthesizing nonhydrolyzable substrates or by conjugating the hydrolyzable ones to long-chain polymers such as dextran. The latter modification would result in multi-ligand binding to endothelial surface enzymes. If these enzymes are associated appropriately with underlying cytoskeletal elements, multi-ligand binding will induce internalization of at least a portion of the bound substrate. Such an hypothesis is worth testing because there is a series of nonpharmacologic, peptide substrates for ACE, whose binding properties are well characterized [38]. The most highly studied of these is [^{3}H]benzoyl-Phe-Ala-Pro (BPAP) [38, 39]. Several others in the series have a higher K_m and V_{max} for ACE than BPAP and also bind to other endothelial receptors as well as ACE [38]. The latter property may actually be desirable, because multiple diseases, drugs and medical procedures reduce ACE activity in the lung, apparently by producing reversible endothelial damage [32, 39]. Because different receptors are lost preferentially in different disease states [32, 39], substances that bind multiple receptors are statistically more dependable as general-purpose carriers. In order for any of these approaches to succeed, both the carrier and the drug must be effectively transported across the contraluminal membrane into the basement membrane and tissues [30, 37] and must not be inordinately metabolized within the endothelial cell itself. The choice of formulation also depends on the purpose of therapy. Modulation of normal lung physiology could be carried out effectively with soluble drug-carrier conjugates. Treatment of tumors and infections will probably require microencapsulated forms of drugs, in order to avoid severe endothelial damage and related pulmonary fibrosis [37, 40]. A number of common drugs also bind to one or more of the preceding receptors and might be useful as carriers for first-order clearance. These include chlorphentermine [41] and desmethylimipramine [42].

Second-order targeting. It is frequently desirable to prolong the residence of a drug within lung tissue, provided the free concentrations are maintained below toxic levels. Even water-soluble agents are retained (nonspecifically) for up to several days if their molecular weights are sufficiently high. Examples include dextrans, nonspecific immunoglobulins used as controls for tumor antibodies [11], and water-soluble fractions of hematoporphyrin derivative [27]. As determined by tissue radioautography, both HPD and specific monoclonal antibodies localize primarily in the extracellular stroma of tumors and are not bound predominately to the tumor cells themselves [11, 27]. Mechanisms of increased hydrostatic pressure, decreased lymphatic drainage, and shedding of tumor cell-surface antigens are thought to be responsible for this problem. Conjugation of methotrexate to poly-L-lysine has markedly increased its tumoricidal activity *in vitro* [43], and studies are in progress to determine its localization *in vivo*. In certain instances, second-order uptake occurs naturally. For example, pulmonary 5-HT accumulates selectively in mast cells under *in vivo* conditions [44]. Also, because of an increased requirement for intracellular iron, neoplastic cells take up transferrin more rapidly than quiescent cells [45]. This may offer a means for semi-selective drug accumulation in tumor cells, provided adequate first-order targeting is achieved. In the context of tumor therapy, mannosylated albumins are both cleared by the lung and concentrated in alveolar macrophages [46]. Conjugates of this carrier with the immunostimulant, muramyl dipeptide, may prove useful in adjuvant therapy of pulmonary neoplasms. In general, when both first- and second-order targeting are required, the device of choice is usually an encapsulation carrier rather than a soluble polymeric drug conjugate.

Bioadhesion targeting. Bioadhesion refers to the combined effects of biophysical trapping and biochemical adherence (Fig. 1). It involves an initial step of physical impedance or occlusive entrapment of particles in microvessels, followed by multi-ligand binding of particle surface material to the endothelium. This second step facilitates transport into the tissue compartment. Bioadhesion targeting is simpler than magnetic methods and is compatible with broader therapeutic applications in a less technical medical environment. It also avoids the deposition of ferromagnetic oxides required for magnetic targeting. To achieve adequate impedance in target capillaries, the particles must be approximately 3–5 μm in diameter. Slight cardiac trapping occurs at this size; however, on a concentration basis, myocardial capture is substantially lower than pulmonary capture.

In selecting an optimal material for surface adherence, we can learn much from the experiments of nature on extravascular migration of neoplastic cells [47], and invasive bacteria [48]. Extravasation of neoplastic cells has been correlated with the presence

of cell-surface receptors for laminin, the major glycoprotein of vascular basement membrane [47]. Also, it has been reported recently that laminin receptors are present in the highly virulent bacterium, *Staphylococcus aureus*, but not in the noninvasive pathogen, *Staphylococcus epidermidis* [48]. Hence, it is possible that laminin receptors and antibodies to laminin will facilitate capillary clearance of drug particles. Recent reports indicate that fibronectin may also be an effective material for promoting the extravascular migration of nonliving particles [49, 50]. Additionally, selected clones of the murine B16 melanoma exhibit very high metastasis to lung, whereas other clones avoid the lung and localize preferentially in liver or brain [51]. This indicates that there are differences in endothelial antigens between pulmonary and extrapulmonary capillaries which might be exploited for pulmonary drug targeting. Preliminary studies in the author's laboratory have given promising results for microvascular bioadhesion using degradable hydrophilic particles coated with either *Ulex europaeus* I lectin [52] or selected heparins (D. F. Ranney, unpublished studies). It is anticipated that the most active heparins will be those with strong complementarity to endothelial heparan sulfates [53, 54]. Upon injection of these particles, biophysical capture occurs preferentially in the lungs, and the particles are enveloped by endothelial processes within approximately 15 min. This renders them anatomically and functionally extravascular. Efficiencies of pulmonary capture vary with particle diameters and coatings. Studies are in progress to optimize these physicochemical parameters. A number of other materials that bind to endothelium and basement membrane molecules also represent promising candidates for adherence. These include antibodies to endothelial factor VIII antigen [55] and type IV collagen of the basement-membrane [56], new monoclonal antibodies that bind selectively to lung microvascular endothelium, but whose binding receptors are not yet characterized [57], glycosylated albumin [58], cationized ferritin [59], and antibodies to inducible procoagulants [60]. The reader is invited to think of additional ones. In general, the preferred molecules are ones that are simplest and least expensive, have the highest stabilities, give the most favorable coupling efficiencies and flexibilities, exhibit the lowest toxicities and suffer the least decrement in binding as a function of disease states, drug regimens, anesthesia, cardiopulmonary bypass, and other medical interventions [32]. Toxic effects of special importance include agglutination of blood cells, activation of complement and coagulation cascades, and elicitation of endothelial and parenchymal-cell toxicity. As one example, a lactose-specific lectin has been described that binds in large numbers to endothelial receptors but has a 3-fold greater affinity for red-cell receptors [61]. If toxicities prove difficult to control for microspheres with exposed binding ligands, thin-film technologies are available to coat these spheres with materials such as BPAP. This would sequester the ligands and permit them to become exposed only after the particles had directly contacted endothelium and the film had been focally hydrolyzed by cell-surface angiotensin converting enzyme. Finally, to prevent adverse effects on pulmonary arterial pressures and lung endothelium [62], microspheres that produce even marginal impedance of flow will have to be administered by selective catheterization of *bronchial* or other systemic arteries.

Concluding remarks. In assessing the impact of various targeting methods on pulmonary drug localization, it appears that a time window will exist between now and the year 2000, during which magnetic microspheres may confer significant therapeutic advantages on several existing drugs. However, as we discover more selective materials for endothelial adherence and learn more about the effects of disease on endothelial (and epithelial) receptors [63], bioadhesion methods will probably approach the targeting efficiencies of magnetic methods today. Biochemical methods will give less efficient pulmonary clearance but, because they are the simplest of the first-order methods, they should enjoy the broadest applications. Second-order targeting is still problematic. It remains to be seen whether efficient binding can be achieved with polyvalent ligands in contrast to the mono- and divalent ones tested to date. Monoclonal antibodies and other affinity agents will probably find their greatest applications in diagnosis rather than therapy. If their cell-binding characteristics can be improved by biochemical modifications, they may be of significant therapeutic value when appropriately encapsulated in a first-order device and targeted to the lung. Three very fruitful areas for future research are the screening of fermentation systems for bacterial adherence products [64] with endothelial specificities, the development of controlled-release devices with improved tissue access and retention [65, 66], and continuous vascular infusion with programmable pumps. The field of pulmonary drug targeting is a promising one and merits adequate resources to support its continued rapid development.

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