Lung uptake of antibodies to endothelial antigens:
key determinants of vascular immunotargeting

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Vascular immunotargeting is a means for a site-selective delivery of drugs and genes to endothelium. In this study, we compared recognition of pulmonary and systemic vessels in rats by candidate carrier monoclonal antibodies (MAbs) to endothelial antigens platelet endothelial cell adhesion molecule (PECAM)-1 (CD31), intercellular adhesion molecule (ICAM)-1 (CD54), Thy-1.1 (CD90.1), angiotensin-converting enzyme (ACE; CD143), and OX-43. Tissue immunostaining showed that endothelial cells were Thy-1.1 positive in capillaries but negative in large vessels. In the lung, anti-ACE MAb provided a positive staining in 100% capillaries vs. 5–20% capillaries in other organs. Other MAbs did not discriminate between pulmonary and systemic vessels. We determined tissue uptake after infusion of 1 μg of 125I-labeled MAbs in isolated perfused lungs (IPL) or intravenously in intact rats. Uptake in IPL attained 46% of the injected dose (ID) of anti-Thy-1.1 and 20–25% ID of anti-ACE, anti-ICAM-1, and anti-OX-43 (vs. 0.5% ID of control IgG). However, after systemic injection at this dose, only anti-ACE MAb 9B9 displayed selective pulmonary uptake (16% vs. 1% ID/g in other organs). Anti-OX-43 displayed low pulmonary (0.5% ID/g) but significant splenic and hepatic uptake (e.g., 18% ID/g of anti-Thy-1.1 in spleen). The lung-to-blood ratio was 5, 10, and 15 for anti-Thy-1.1, anti-ACE, and anti-ICAM-1, respectively. PECAM antibodies displayed low pulmonary uptake in perfusion (2% ID/L) and in vivo (3–4% ID/L). However, conjugation with streptavidin (SA) markedly augmented pulmonary uptake of anti-PECAM in perfusion (10–54% ID, depending on an antibody clone) and in vivo (up to 15% ID/L). Therefore, ACE-, Thy-1.1-, ICAM-1-, and SA-conjugated PECAM MAbs are candidate carriers for pulmonary targeting. ACE MAb offers a high selectivity of pulmonary targeting in vivo, likely because of a high content of ACE-positive capillaries in the lungs.

SITE-SELECTIVE DELIVERY of drugs or genes to endothelial cells may provide more effective and specific means for prophylaxis or therapies of pathological conditions associated with endothelial dysfunction, such as hypertension, thrombosis, atherosclerosis, acute respiratory distress syndrome, cancer, inflammation, and diabetes (3, 17, 23). Endothelial cells display antigenic and functional heterogeneity in different tissues and organs, either normal or pathologically altered (22, 47, 53, 63). Even in the same organ, endothelial cells may normally exhibit a significant heterogeneity in different segments, e.g., venous vs. arterial (21, 33). It is tempting to postulate that endothelial heterogeneity may be used for more precise targeting of drugs to a defined organ or vascular area. These considerations provide a rationale and framework for the vascular immunotargeting strategies using antibodies directed against endothelial surface determinants as carriers for site-selective drug delivery.

Importantly, pulmonary endothelium represents a privileged vascular target because lungs receive the first passage of the whole cardiac output of venous blood and possess a high density of capillaries (~30% of the total endothelium in the body belongs to the pulmonary vasculature; see Ref. 19). Therefore, even antibodies that do not discriminate between pulmonary and systemic endothelium may accumulate in the lung after intravascular injection. In support of this notion, native and modified antibodies to angiotensin-converting enzyme (ACE; see Refs. 11 and 43), thrombomodulin (TM; see Ref. 31), intercellular adhesion molecule (ICAM)-1 (48), platelet endothelial cell adhesion molecule (PECAM)-1 (35, 42), and a nonidentified caveola-associated antigen (57) recognize endothelium in vivo, accumulate in the lungs, and may serve as carriers for drug/gene targeting to the endothelium.

None of these antibodies, however, qualify as universal or ideal carriers, since every pathological setting presents unique requirements for a drug delivery system. The need for different carriers to design delivery...
systems adequately serving various therapeutic aims justifies studies of endothelial recognition by antibodies and the quest for new potentially useful carriers. However, a systematic characterization of some potentially useful carriers in terms of their binding to endothelium in the lungs and in extrapulmonary blood vessels is missing in the literature. The goal of the present work was to characterize distribution of the target endothelial antigens in the vasculature of laboratory animals and correlate this information with the data on biodistribution of radiolabeled monoclonal antibodies (MAbs) after systemic injection in vivo.

Theoretically, success of vascular immunotargeting strategies depends on tissue distribution of surface endothelial antigens, their accessibility to carrier MAbs, and affinity of the MAbs. Therefore, evaluation of these parameters is an important component of immunotargeting strategies. To characterize distribution of the antigens in organs, including lungs, we stained rat tissue sections with MAbs and graded semiquantitatively intensity of immunostaining in various vascular areas. To estimate accessibility of the endothelial antigens to circulating MAbs, we determined uptake of radiolabeled MAbs in isolated perfused rat lungs. To evaluate tissue selectivity of the targeting and evaluate potential applicability of the MAbs as drug carriers, we characterized tissue distribution of radiolabeled MAbs in rats after intravascular injection in vivo.

We studied MAbs directed against the following surface endothelial antigens: CD31 (anti-PECAM-1 MAb 164.1), CD54 (ICAM-1 MAb 1A29), CD90.1 (anti-Thy-1.1 MAb OX-7), CD143 (anti-ACE MAb 9B9), and the murine MAb against rat endothelium (MRC OX-43). We also determined the affinity of several PECAM antibodies and their uptake in the lungs. Our results indicate that antibodies directed against ACE, ICAM-1, Thy-1, and PECAM modified with streptavidin (SA) might serve as carriers for vascular immunotargeting to different vascular areas, including lung, heart, and spleen. Of specific interest in the context of lung biology and pathology, anti-ACE MAbs offer more selective pulmonary targeting in vivo than other candidate carriers tested in this work, most likely because of a very high content of ACE in the pulmonary capillary endothelium.

**MATERIALS AND METHODS**

**Materials.** IODO-GEN was obtained from Pierce (Rockford, IL), and Na[125I] was from Amersham (Arlington Heights, IL). Dimethyl formamide, TCA, and normal mouse IgG were purchased from Sigma (St. Louis, MO). SA and 6-biotinylaminocaproic acid N-hydroxysuccinimide ester (long-arm biotin ester) were purchased from Calbiochem (San Diego, CA).

**Antibodies.** Properties of the anti-ICAM-1 (CD54) MAb (clone 1A29) were described previously (48, 59). This MAb was purchased from PharMingen (San Diego, CA). Mouse anti-rt endothelium MAb, clone MRC OX-43 (54), was purchased from Serotec (Oxford, UK). Mouse MAb against rat CD90.1 (Thy-1.1; clone OX-7) was from PharMingen. Properties of the anti-ACE MAbs (clones 9B9 and 5F1) have been described previously (9–11). Anti-ACE 9B9 cross-reacts with human, rat, and cat ACE, whereas MAb 5F1 does not cross-react with rat ACE and therefore served as a negative control in immunohistochemistry studies. The following antibodies directed against PECAM-1 (CD31) were used in the study: MAb clone 164.1 (along with a purified chimera protein CD31) was kindly provided by Dr. P. Newman (Blood Research Institute, Milwaukee, WI); MAb clones 4G6 and 37 and polyclonal rabbit antibody “Houston” were kindly provided by Dr. S. M. Albeda (University of Pennsylvania); MAb clone 6E4 was kindly provided by Drs. T. Vlasik and A. Mazurov (Russian Cardiology Research Center, Moscow); MAb clone 62 was provided by Dr. M. Nakada (Centocor; see Ref. 44). Mouse MAb 164.1 was produced using rat PECAM-1 isoform as an immunogen. All other monoclonal and polyclonal antibodies have been produced using human PECAM-1 as an immunogen; however, some clones cross-reacted with rat PECAM isoform.

**Immunostaining of rat tissues with anti-endothelial antibodies.** Immunoenzymatic detection of ACE and other endothelial antigens was performed by the alkaline phosphatase anti-alkaline phosphatase (APAAP) technique (5), adapted for ACE staining as described (37). In brief, frozen tissue sections were air-dried and fixed in acetone for 10 min at room temperature. Sections were incubated with the primary MAb and then with rabbit anti-mouse Ig (1:40; DAKO) supplemented with a 1:750 dilution of reconstituted lyophilized rat serum (Dianova). This was followed by incubation with the APAAP complex (1:50; DAKO). Each step lasted 30 min at room temperature. Samples were thoroughly washed in Tris- and HCl-buffered saline (pH 7.6) between steps. Alkaline phosphatase substrate reaction with new fuchsin (100 µg/ml) and levamisole (400 µg/ml) was performed for 20 min at room temperature. Sections were counterstained with hematoxylin and mounted in gelatin. Before the systematic study of endothelial antigen distribution, different dilutions of all MAbs were tested on different native tissues to determine the optimal MAb concentration, which was between 1 and 10 µg/ml. Further increase of these concentrations did not increase the maximal intensity of endothelial immunostaining, suggesting saturation of all antigenic sites.

To estimate the level of endothelial antigen expression within the different vessels, a semiquantitative analysis of immunostaining was performed as previously described (25, 52). Recently, this approach was successfully employed for the quantification of ACE expression in kidneys in humans (37). The predominant expression pattern of endothelial antigens was estimated for each vascular segment using the following increments of staining intensity: negative (−), weak (+), moderate (++), or strong (+++). PECAM-1 (CD31) is expressed at high levels in endothelial cells in different vessels (a "pan-endothelial antigen"; see Ref. 49). Accordingly, +++ characterized an intensity of vascular staining with antibodies as strong as the immunostaining with the anti-PECAM (37). Previously, experiments with various concentrations of various anti-ACE MAbs showed that an increase in one immunohistochemical scoring point was equivalent to a 10-fold increase in immunoreactive sites (Franke, unpublished observations). Tissue sections were inspected by two morphologists (Franke and Pauls), and the immunoreactivity score and mean expression were assigned independently without substantial differences. More than 300 cryostat sections of the vascular tree in the main organs of six rats were analyzed in the study.

**Radiolabeling of antibodies.** In a pilot study, we determined the optimal conditions for radiolabeling of antibodies by iodinating MAb 9B9 using IODO-GEN-coated tubes
(Pierce), IODO-beads (Pierce), or Bolton-Hunter reagent (Amersham). The results of this comparison, shown in Table 1, indicate that the IODO-GEN method provides the best results in terms of effectiveness of radiolabeling and binding capacity of the antibody. Based on this result, we used the IODO-GEN method for radiolabeling of all antibodies used in the study. Briefly, 100 μg of protein were incubated with 100 μCi of Na\(^{125}\)I for 30 min on ice. The excess iodine was removed by gel filtration on a PD-10 (Sephadex G-25) mini-column (Pharmacia, Uppsala, Sweden). Analysis with TCA precipitation of radiolabeled proteins showed that free iodine content in the labeled antibodies did not exceed 5%.

**Analysis of binding of radiolabeled PECAM antibodies to immobilized CD31.** Plastic wells of “Microtest” plates were coated with human CD31 by overnight incubation at 4°C with 100 ng in BBS (pH 8.1). After elimination of nonbound CD31, wells were incubated with BSA to block nonspecific binding sites. Wells coated with BSA without CD31 served as a control for the specificity of binding. \(^{125}\)I-labeled antibodies were incubated in the wells for 1 h at room temperature. After elimination of nonbound materials, radioactivity in the wells was determined in a gamma counter.

**Perfusion of isolated rat lungs.** Male Sprague-Dawley rats weighing 170–200 g were anesthetized with pentobarbital sodium (50 mg/kg ip). Lungs were isolated and perfused with a recirculating buffer as previously described (18). Briefly, the trachea was cannulated, and the lungs were ventilated with a humidified gas mixture (Aireco, Philadelphia, PA) containing 5% CO\(_2\) and 95% air. Ventilation was done with an SAR-830 rodent ventilator (CWE) at 60 cycles/min, a tidal volume of 2 ml, and an end-expiratory pressure of 2 cmH\(_2\)O. The thorax was then opened, and a cannula was placed in the main pulmonary artery through the transected heart. The lungs were isolated from the thorax and transferred to a water-jacketed perfusion chamber maintained at 37°C. There was no interruption of ventilation during this process, and interruption of the lung perfusion was <5 s. Perfusion through the pulmonary artery was maintained by a peristaltic pump at a constant flow rate of 10 ml/min. The perfusate was Krebs-Ringer buffer (KRB, pH 7.4) containing 10 mM glucose and 3% fatty acid-free KRB-BSA solution. The perfusate was filtered through a 0.4-μm filter before perfusion to eliminate particles. One microgram of radiolabeled MAb was added to the perfusate. After 1 h of perfusion with the radiolabeled protein, the lungs were perfused with a single-pass perfusion for 5 min with perfusate that did not contain radiolabeled protein to eliminate nonbound radioactivity. At the end of experiment, the lungs were removed from the chamber, rinsed with saline, and blotted with filter paper; the lung wet weight was determined, and its radioactivity was measured in a gamma counter and expressed as a percentage of perfused radioactivity per lung. The radioactivity associated with parts of the perfusion system was determined in a prototype experiment. This parameter did not exceed 0.01% of the total radioactivity added to the perfusate. Accordingly, this value has been ignored in the analysis of the results.

**In vivo administration of radiolabeled antibodies and biodistribution studies.** Male Sprague-Dawley rats were injected with \(^{125}\)I-labeled MAb via the tail vein under halothane anesthesia. A design, protocol, and analysis of a typical experiment have been described previously (11). Briefly, animals were killed 2 h postinjection, and the internal organs were dissected, washed with saline, blotted dry, and weighed. Tissue radioactivity in organs was determined in a gamma counter; 1 ml of blood, a piece of liver (~1 g), and the entire nonhomogenized lung, spleen, kidney, and heart were used for measurement.

The results of \(^{125}\)I measurements in the organs were used to calculate three parameters characterizing related yet different aspects of \(^{125}\)I-MAb biodistribution and targeting. First, the percentage of the injected dose in an organ (%ID) characterizes a total value of antibody uptake in an organ. This parameter shows biodistribution and effectiveness of antibody targeting. Second, the percentage of the injected dose per gram of tissue (%ID/g) permits comparisons of uptake of the antibodies in different organs. Without such normalization, uptake in larger organs (e.g., liver) may look extremely large, even when compared with a very specific and effective uptake in a relatively small organ (e.g., lung or heart). Therefore, this parameter evaluates tissue selectivity of an antibody uptake. Importantly, this parameter also permits comparison of the data obtained in different animal species. These two parameters (%ID and %ID/g) provide different absolute values in many organs (including lung, which is not 1 g exactly in rats), as reflected in the difference between values in Table 4 and Fig. 3. Third, the ratio between percent ID per gram in an organ and that in blood gives the localization ratio (LR). This parameter compensates for a difference in the blood level of circulating radiolabeled antibody (e.g., due to the different rate of uptake by clearing or target organs). Therefore, LR allows a more objective comparison of targeting between different carriers, which have different rates of blood clearance.

**RESULTS**

**Immunohistochemical characterization of antigen distribution in rat tissues.** In the first part of this work, we characterized immunostaining of ACE (CD143), Thy-1.1 (CD90.1), ICAM-1 (CD54), PECAM-1 (CD31), and rat OX-43 antigen in rat organs using MAbs employed for in vivo studies (see below). The aims of this study were to 1) compare binding of different MAbs in the organs under identical conditions and evaluate the total content of MAb binding sites in the organs; 2) visualize cell types recognized by MAbs; and 3) reveal potential heterogeneity of the MAbs binding to endothelial cells in different types of blood vessels in the vasculature. For maximal epitope preservation, we used fresh frozen tissues for immunostaining sections. A preliminary study showed that acetone fixation employed in the study, a standard procedure for tissue...
preparation for immunostaining using fresh frozen tissues, does not alter the epitopes.

All tested MAbs except anti-PECAM MAbs 6E4 and 4G6 labeled endothelial cells in the rat tissues (Table 2). This result indicates that they cross-react with the rat isofrom of human counterpart antigens and therefore are suitable for the following experiments in perfusion of isolated rat lungs and in vivo. When incubated with the tissue sections, MAbs also displayed binding to other cell types than the endothelium in certain organs. For example, Thy-1.1 was found in lymphocytes in the spleen (Fig. 1E), PECAM-1 was found in macrophages and platelets in the spleen (Fig. 1F), ICAM-1 was found in macrophages (data not shown), and ACE was found in the epithelial cells in the kidneys and epididymis (data not shown).

Studied MAbs displayed different staining patterns in the endothelium in systemic and peripheral vessels of a different caliber (reactivity in the pulmonary vasculature is described below). For example, anti-Thy-1.1 MAb displayed positive staining in the capillary but not in the arterial and venous endothelium (Figs. 1A and 2D). In contrast, anti-ACE MAb displayed positive staining in arterial endothelial cells (Fig. 1B), whereas capillary endothelium staining was sparse or even negative in most organs, except the lung. Antibodies against ICAM-1 and PECAM-1 displayed a more homogeneous immunostaining pattern in endothelial cells in the vessels of different caliber throughout the vascular system (Fig. 1C). This pattern of anti-ICAM and anti-PECAM MAbs correlates well with a relatively homogeneous distribution of their antigens in vasculature in diverse animal species described in the literature (Refs. 14 and 49, respectively). Table 2 summarizes the data on the distribution of the studied antigens in endothelial cells in different types of vessels.

Because capillaries represent the most extended vascular surface area in many target organs, we specifically characterized the MAb immunostaining pattern in the capillaries in different organs. In this type of blood vessels, ACE antigen displayed the most striking pulmonary selectivity: 100% of alveolar capillaries were strongly labeled by the anti-ACE MAb 9B9 (Fig. 2A). In sharp contrast, hepatic and renal capillaries were negative, and only 5–20% of the capillaries in other organs were positive for anti-ACE MAb immunostaining. Other tested antibodies also showed labeling of pulmonary capillary endothelial cells (Fig. 2, B–E). However, their antigens were well presented in the capillaries of other organs as well, except for a relatively weak immunostaining of ICAM-1 in cardiac and PECAM-1 in splenic capillaries (Table 3).

Uptake of 125I antibodies in the isolated perfused rat lung. Exposure of tissue sections to antibodies for a sufficient time allows binding to a maximal number of tissue epitopes, both intra- and extracellular. Therefore, immunochemical study does not provide an insight into the antigen’s ability to harbor ligands circulating in the bloodstream. To address this important issue directly and estimate accessibility of the endothelial antigens to circulating antibodies in the absence of potentially confusing systemic and blood effects, we measured the uptake of 125I-MAbs in isolated rat lungs perfused with blood-free buffer. Figure 3 shows that anti-ICAM-1 MAb 1A29, anti-ACE MAb 9B9, and anti-endothelial MAb MRC OX-43 accumulated in rat lungs at a similar extent, ranging from 20 to 25% ID. noteworthy, pulmonary uptake of anti-Thy-1 MAb OX-7 was extremely high (45% ID). Control 125I-labeled IgG did not accumulate in the perfused rat lungs (0.4 ± 0.04% ID), thus confirming the specificity of accumulation of the radiolabeled anti-endothelial 125I-MAbs. Anti-PECAM MAb 164.1 demonstrated low pulmonary uptake (1.9 ± 0.8% ID). The issue of PECAM antibody affinity and targeting is addressed separately in Binding of PECAM MAbs to purified CD31 antigen and to pulmonary endothelium.

Distribution of 125I-MAb in the tissues after intravenous injection in rats in vivo. Table 4 shows the data for tissue distribution of 125I-MAbs 2 h after intravenous injection in intact anesthetized rats. The blood level of control IgG was close to 3% ID/g. Taking into account that, in an average-sized rat (250 g), total blood volume is ~15–18 ml, this result indicates that ~50% of injected IgG circulates in the bloodstream. The level of anti-endothelial 125I-MAbs in the blood was significantly lower than that of the control IgG. This result likely reflects their binding to the endothelium (and, perhaps, other target cells in the tissues accessible to the bloodstream), leading to depletion of the circulating pool.

Control 125I-IgG did not accumulate in any organ. Absolute values of IgG uptake in the tissues were significantly lower than its blood level. Therefore, the IgG tissue-to-blood ratio (LR) was <0.5 in all tissues.

Table 2. Endothelial reactivity pattern of different MAbs within the systemic vasculature of the rat except the vessels of lung

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Antibody</th>
<th>Aorta</th>
<th>Arteries</th>
<th>Arterioles</th>
<th>Capillaries</th>
<th>Veins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unknown</td>
<td>MRC OX-43</td>
<td>+++</td>
<td>+++/+</td>
<td>+++/+</td>
<td>+++/+</td>
<td>+++</td>
</tr>
<tr>
<td>PECAM-1 (CD31)</td>
<td>164.1</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++/-</td>
<td>+/++</td>
</tr>
<tr>
<td>Thy-1.1 (CD90.1)</td>
<td>OX-7</td>
<td>–</td>
<td>–</td>
<td>–/+</td>
<td>+/+/-</td>
<td>–/–</td>
</tr>
<tr>
<td>ICAM-1 (CD54)</td>
<td>1A29</td>
<td>+++</td>
<td>+/+/-</td>
<td>+/-</td>
<td>+/–</td>
<td>+/–</td>
</tr>
<tr>
<td>ACE (CD143)</td>
<td>9B9</td>
<td>+++</td>
<td>+++</td>
<td>+/–</td>
<td>–/+</td>
<td>+/–</td>
</tr>
</tbody>
</table>

PECAM, platelet endothelial cell adhesion molecule; ICAM, intercellular adhesion molecule. The degree of immunoreactivity is scored from – (no reactivity) to +++ (strong reactivity), whereby dash (/) indicates variations in immunoreactivity within the same types of vessels. Note the relative homogeneous endothelial distribution of CD31, e.g., compared with CD90.1.
including the lungs (LR <0.3). None of the tested antibodies accumulated in the intestine. Renal uptake was low for all antibodies, although anti-PECAM-1 and anti-Thy-1.1 MAbs displayed a slightly higher renal uptake than control IgG. Anti-endothelial MAb MRC OX-43 demonstrated a marked uptake in the spleen and significant uptake in the heart, whereas its pulmonary accumulation was negligible.

The data in Table 4 are expressed as percentage of the injected dose per gram to allow comparison between different organs. To evaluate total uptake in tissues, one has to multiply the data on organ weight (e.g., on average, 15 and 12 g for blood and liver). Therefore, it is relatively easily to take into account the blood pool, ranging from 50 to 20% ID (IgG and anti-ACE, respectively), and hepatic uptake, ranging from 40 to 15% ID (anti-ICAM and anti-ACE, respectively). However, as seen with tracing of radiolabeled proteins in vivo, the balance of injected tracers in major organs and blood does not usually sum up to 100% ID and averages 60–80% ID for many MAbs. The main reason is that it is difficult to accurately determine uptake in skin, muscles, and other nonmeasured parts of the body.
Many MAbs employed in the study displayed the significant accumulation in the lungs. Notably, among other tested antibodies, anti-ACE MAb 9B9 displayed the highest absolute value (16% ID/g) and selectivity of the pulmonary uptake, with no detectable uptake in other organs. Anti-ICAM-1 and anti-Thy-1.1 MAbs displayed moderate and modest pulmonary uptake (4 and 6% ID/g) and also accumulated in liver and spleen. Anti-Thy-1.1 MAb OX-7 displayed a remarkably high uptake in the spleen (18% ID/g). Uptake in the nonpulmonary targets (correlating with a high content of ICAM-1 and Thy-1.1 in the nonpulmonary capillaries; Table 3) led to a pronounced depletion of the blood pool of 125I-anti-Thy-1.1 and 125I-anti-ICAM-1. As a result, pulmonary LR of these 125I-MAbs was close to 5 and 15, respectively. Taken together, the results presented indicate that antibodies against ICAM-1 and Thy-1.1 accumulate promiscuously in many organs, including the lungs, whereas anti-ACE accumulates selectively in the lungs.

Table 3. Distribution of antigen-positive capillaries in pulmonary vs. systemic circulation

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Antibody</th>
<th>Lung</th>
<th>Liver*</th>
<th>Kidney</th>
<th>Spleen</th>
<th>Heart</th>
<th>Intestine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unknown</td>
<td>MRC OX-43</td>
<td>100</td>
<td>20</td>
<td>100</td>
<td>ND</td>
<td>100</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>PECAM-1 (CD31)</td>
<td>164.1</td>
<td>100</td>
<td>100</td>
<td>50</td>
<td>10</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Thy-1.1 (CD90.1)</td>
<td>OX-7</td>
<td>80</td>
<td>0</td>
<td>50</td>
<td>30</td>
<td>50</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>ICAM-1 (CD54)</td>
<td>1A29</td>
<td>100†</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>ACE (CD143)</td>
<td>9B9</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>20</td>
<td>10</td>
<td>5</td>
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<tr>
<td></td>
<td></td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

The percentage of immunoreactive endothelial cells in the capillary system of different organs is given in numerical values; the intensity of antigen expression is indicated by score values from − (no expression) to +++ (strong expression). *Sinusoids of liver parenchyma, which represent >99% of the endothelial surface of liver. ND, not determined (because of strong background immunoreactivity concerning interstitial and inflammatory cells). †Difficult to assess in alveoli of rat lung because CD54 was also found in regular alveocytes.
Binding of PECAM MAbs to purified CD31 antigen and to pulmonary endothelium. Figure 3 shows that anti-PECAM MAb 164.1 displayed a relatively modest, nonselective uptake in the lungs and other organs in vivo (3.5% ID/g in the lung, LR >10). Therefore, MAb 164.1 displayed slightly higher pulmonary uptake in vivo than in the perfused rat lungs (2% ID). The blood plasma volume in rats (6 ml) is seven times smaller than that of the perfusion buffer (45 ml). Therefore, an apparent contradiction between MAb 164.1 pulmonary uptakes in vivo and in perfusion can be reconciled based on the assumption that the affinity of the MAb 164.1 clone is a limiting factor. In general, the affinity of MAbs binding to endothelial antigens is a critically important parameter of the targeting. Capitalizing on the availability of purified human CD31 (kindly provided by Dr. P. Newman), we performed a more systematic study of the binding profile of different anti-PECAM clones.

Figure 4 shows typical binding curves of radiolabeled MAb (MAb 6E4; A, and MAb 62; B) and polyclonal antibodies (rabbit Ab Houston) to immobilized CD31. The bottom curves in Fig. 4; C, represent binding to control wells coated with albumin. Binding of 125I-IgG to CD31-coated wells did not differ from binding of 125I-anti-PECAM to albumin. These controls help to appreciate a high specificity of anti-PECAM binding to CD31. To characterize the affinity of PECAM antibodies, the Scatchard analysis of the binding curves (linear regression between bound ligand and the bound-to-free ratio) has been performed. Figure 4, insets, shows results of this regression and indicate that binding curves of all anti-PECAM clones display a fairly good linearization pattern, permitting determination of their affinity constants (Kd).

Table 5 shows that Kd values determined for five different clones of anti-PECAM varied from relatively high (5 and 10 nM for MAbs 37 and 62) to relatively low (250 nM for polyclonal antibody Houston). However, despite significant variations in affinity, all tested PECAM antibodies displayed relatively low uptake in the perfused rat lungs (2.0%–2.5% vs. 0.6% ± 0.1% ID/g for control IgG) and in rat lungs in vivo (2.5%–3.6% ID/g). In the previous study, we observed that coupling to SA augments binding and internalization of biotinylated PECAM antibodies by endothelium (42). In good agreement with this early observation, experiments in perfused rat lungs showed that conjugation with SA augmented pulmonary uptake of biotinylated Ab Houston and MAb 62 to 40–50% ID/g. SA had no effect on pulmonary uptake of biotinylated Ab Houston and MAb 62 to 40–50% ID/g. In the previous study, we observed that coupling to SA augments binding and internalization of biotinylated PECAM antibodies by endothelium (42). In good agreement with this early observation, experiments in perfused rat lungs showed that conjugation with SA augmented pulmonary uptake of biotinylated Ab Houston and MAb 62 to 40–50% ID/g. SA had no effect on pulmonary uptake of biotinylated control IgG. Conjugation of biotinylated MAb 164.1 with SA markedly augmented its pulmonary uptake in perfused rat lungs (10% ID/g) yet had no effect on pulmonary uptake in

Table 4. Biodistribution of 125I-MAbs in rat after systemic injection

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Antibody</th>
<th>Blood</th>
<th>Lung</th>
<th>Kidney</th>
<th>Spleen</th>
<th>Heart</th>
<th>Intestine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unknown</td>
<td>MRC OX-43</td>
<td>1.12±0.34</td>
<td>0.47±0.12</td>
<td>0.69±0.22</td>
<td>0.93±0.35</td>
<td>6.67±2.09*</td>
<td>1.89±0.42*</td>
</tr>
<tr>
<td>PECAM-1 (CD31)</td>
<td>164.1</td>
<td>0.27±0.05</td>
<td>3.55±0.78*</td>
<td>2.93±0.49*</td>
<td>1.47±0.38*</td>
<td>1.74±0.26*</td>
<td>1.30±0.59*</td>
</tr>
<tr>
<td>Thy-1.1 (CD90.1)</td>
<td>OX-7</td>
<td>0.77±0.25</td>
<td>3.73±1.10*</td>
<td>1.73±0.33*</td>
<td>1.57±0.27*</td>
<td>18.13±4.01*</td>
<td>0.14±0.03</td>
</tr>
<tr>
<td>ICAM-1 (CD54)</td>
<td>A129</td>
<td>0.34±0.02</td>
<td>5.52±1.99*</td>
<td>2.79±0.44*</td>
<td>0.80±0.06</td>
<td>3.22±0.60*</td>
<td>0.49±0.14</td>
</tr>
<tr>
<td>ACE (CD143)</td>
<td>9B9</td>
<td>1.67±0.62</td>
<td>15.65±5.38*</td>
<td>0.65±0.18</td>
<td>0.47±0.14</td>
<td>0.48±0.16</td>
<td>0.45±0.13</td>
</tr>
<tr>
<td>Control</td>
<td>5F1</td>
<td>3.79±0.46</td>
<td>0.87±0.21</td>
<td>0.94±0.20</td>
<td>1.04±0.07</td>
<td>0.46±0.11</td>
<td>0.78±0.09</td>
</tr>
</tbody>
</table>

Data are means ± SD; n = 3 experiments. Control, 125I-mouse IgG. Units are %ID/g tissue. Rats were injected via tail vein with 125I-MAbs (300,000 counts/min, 1 μg). After injection (2 h), animals were killed, internal organs were dissected, washed with saline, and weighed; and radioactivity in organs was measured in a Rack-Gamma counter. *Values of accumulation statistically significant from control IgG.
vivo. However, MAb 62 conjugated with SA displayed a very high pulmonary uptake after injection in intact rats. The absolute value of SA-MAb 62 uptake attained 15% ID/g, close to that of anti-ACE MAB 9B9 (see Fig. 3). Therefore, the anti-PECAM-SA conjugate represents a good candidate for vascular immunotargeting to pulmonary endothelium.

DISCUSSION

Pulmonary endothelium represents an important and privileged target for site-specific delivery of therapeutic agents or genes. Immunotargeting strategies are based on conjugation (chemically or via gene engineering) of a drug (enzyme, gene) with carrier antibodies that recognize specific antigens on the surface of the target cell (51). Antibodies directed against surface endothelial antigens represent potential carriers for the targeting of drugs or genes to the pulmonary endothelium. For example, TM antibody recognizes endothelial cells in vitro and in vivo, accumulates in the murine lung after intravascular injection (31), and is capable of delivery of liposomes or genetic material to the pulmonary endothelium (36, 60). The therapeutic applicability of anti-TM is, however, restricted by the intracellular degradation of internalized TM ligands (26, 41) and by suppression of the anticoagulant function of TM by anti-TM (16). Schnitzer (57) reported the pulmonary accumulation of an antibody recognizing the nonidentified caveola-associated antigen in rats in vivo. However, both the function of this antigen and potential effects of the antibody remain to be characterized more rigorously to evaluate their therapeutic potential. Antibodies recognizing surface adhesion molecules such as PECAM-1, ICAM-1, E-selectin, P-selectin, and vascular cell adhesion molecule-1 also represent a class of potentially useful carriers (32, 48, 58), although in vivo characterization of these antibodies requires more rigorous study.

To further extend and better characterize the list of potential antibody carriers, in this work we studied binding of antibodies against ACE, PECAM-1, ICAM-1, and Thy-1.1 to their antigens in tissue sections, in the perfused rat lungs, and in vivo. The primary goal of this work was to directly compare different MAb carriers in standard experimental models. To accomplish this goal, we employed certain specific experimental conditions and parameters. First, to facilitate evaluation of total binding of MAbs in the tissues, we did not inflate lungs harvested for immunochemical study. A more compact tissue of hypoventilated lung permitted us to compare the gross binding of different MAbs in comparative study. This approach might preclude a more precise localization of the antigens in specific cell types and compartments (e.g., intracellular vs. surface). Electron microscopy or use of the avidin-biotin couple could, theoretically, provide an insight into surface localization and accessibility of the antigens [e.g., see previous publications on this subject (40, 42)]. However, electron microscopy data are difficult to collect and analyze quantitatively in a significant number of animals. On the other hand, accessibility to biotinylating agents (small molecules, ~400 Da) may be higher than that to large antibody carriers (IgG, 180,000 Da). Therefore, we believe that, in the context

Fig. 4. Binding of different anti-PECAM MAbs to immobilized human CD31. Radiolabeled anti-PECAM antibodies were incubated in the wells coated with CD31 (○) or albumin (□) for 1 h at room temperature. The binding was determined after extensive washing of nonbound antibodies and is expressed as means ± SD (n = 3). Insets show linearization of the CD31 binding curves in Scatchard graphs (y-axes show the bound-to-free ratio, x-axes show bound MAb).
of drug/gene delivery, a direct measurement of the tissue uptake of radiolabeled MAbs provides the most accurate information about accessibility of the target antigens to potential carriers.

Analysis of immunotargeting (i.e., 125I-MAb uptake in different organs) can be performed in several ways, including normalization of MAb uptake per surface vascular area in the organs. However, literature on the vascular surface within the same organ is identical for all studied antibodies. Therefore, analysis of pulmonary uptake of nonspecific control IgG and antibodies directed against pan-endothelial antigens (i.e., ICAM-1) implies that a high pulmonary uptake of anti-ACE likely reflects a specifically high presentation of ACE in the pulmonary capillaries.

ACE is a transmembrane ectopeptidase localized on the luminal surface of the vascular endothelium (2, 55). Anti-ACE MAb 9B9 recognizes rat and human ACE and accumulates in rat lung with high specificity and effectiveness (20–30% ID; see Ref. 11). Recently, we published results of a semiquantitative immunohistochemical study that described a very heterogeneous distribution of ACE in human endothelial cells along the vascular tree in humans. Although the endothelium in small arteries and arterioles displayed a strong ACE expression, the endothelium in aorta, large arteries, and veins displayed poor expression or complete lack of ACE. In the extrapulmonary capillaries, only 10–20% were positive by anti-ACE staining, whereas 100% of pulmonary capillaries displayed strong immunoreactivity with anti-ACE MAb (20, 25, 37).

Results of the present study indicate that, in contrast to the human endothelium, rat endothelium displays more homogeneous and strong ACE expression in large vessels of all types, in either arteries or veins. However, anti-ACE reactivity in rat capillaries and small veins was similar to that seen in human tissues; ACE staining was positive in ~10% of capillary endothelial cells in extrapulmonary tissues, whereas 100% of pulmonary capillaries expressed ACE (Table 3). In good agreement with ACE distribution in the rat vasculature, the radiolabeled MAb 9B9 displayed a tissue-selective accumulation in the lung (Table 4 and Fig. 3).

Table 5. Binding of 125I-PECAM antibodies to purified CD31 and their pulmonary uptake in perfused rat lungs and in vivo

<table>
<thead>
<tr>
<th>Antibody</th>
<th>IgG</th>
<th>Antibody Houston</th>
<th>MAb 62</th>
<th>MAb 164.1</th>
<th>MAb 6E4</th>
<th>MAb 37</th>
<th>MAb 4G6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kₐ, nM</td>
<td>NA</td>
<td>250*</td>
<td>10*</td>
<td>ND</td>
<td>20</td>
<td>5*</td>
<td>30*</td>
</tr>
<tr>
<td>b-MAb uptake in IPL, % ID/g</td>
<td>0.6±0.1</td>
<td>2.5±0.8*</td>
<td>2.3±2.1*</td>
<td>2.1±0.3*</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>SA/b-MAb uptake in IPL, % ID/g</td>
<td>0.5±0.2</td>
<td>53.5±9.3*</td>
<td>39.5±3.1*</td>
<td>10.5±1.4*</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>b-MAb uptake in vivo, % ID/g</td>
<td>2.3±0.7</td>
<td>ND</td>
<td>3.6±1.1*</td>
<td>2.6±0.2*</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>SA/b-MAb uptake in vivo, % ID/g</td>
<td>2.1±1.1</td>
<td>ND</td>
<td>14.5±2.9*</td>
<td>1.4±0.5*</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Values are means ± SE. SA, streptavidin; IPL, isolated perfused lungs. All antibodies have been produced using human PECAM-1 as an immunogen. Affinity of binding to PECAM-1 varies from relatively high (MAb 37 and 62) to low (polyclonal antibody Houston). †Binding affinity of MAb 4G6 and MAb 37 was determined by direct RIA similar to other antibodies, except that human umbilical vein endothelial cells were used as an antigen instead of purified CD31 (binding was performed at 4°C). Antibodies MAb 6E4 and MAb 4G6 did not display cross-reactivity with the rat PECAM isoform. Please note that biotinylated (b) antibodies were used in this study; a minor covalent marker might be. Different antigens, including ACE and PECAM-1, have been proposed as pan-endothelial markers for the sake of interpretation of biodistribution of radiolabeled antibodies in vivo (48, 61). However, an average or “generic” endothelial cell is a constituency that probably does not exist in a real vasculature. For example, it is difficult to exclude that endothelial cells in different vascular areas (even in the same organ) can possess different numbers of copies of PECAM-1. A systematic, morphometric study of surface density of PECAM in the vasculature has yet to be performed. Because the goal of our research is to develop a drug delivery strategy, we used more standard, conventional pharmacokinetic parameters (%ID and %ID/g and LR) that permit comparison between target and nontarget uptake of different agents. From the drug delivery standpoint, percent ID and percent ID per gram characterize the concentration of the delivered drug in target and nontarget organs and therefore permit comparison of local and systemic effects. Speculatively, a relatively high pulmonary uptake of any given antibody could reflect the simple fact that the lung vascular area is larger than that in other organs. However, the vascular surface within the same organ is identical for all studied antibodies. Therefore, analysis of pulmonary uptake of nonspecific control IgG and antibodies directed against pan-endothelial antigens (i.e., ICAM-1) implies that a high pulmonary uptake of anti-ACE likely reflects a specifically high presentation of ACE in the pulmonary capillaries.
lum. The fact that radiolabeled ACE inhibitors (small molecules, 500 Da) accumulate in isolated perfused lung (29) and in whole animals (28) with the same efficacy as the MAb 9B9 (IgG, 170,000 Da) suggests that the accessibility of the ACE epitope for MAb 9B9 is close to ideal. Endothelial cells internalize anti-ACE MAb 9B9 (40). Thus anti-ACE may be useful for intra-vascular delivery. Anti-ACE MAbs cause no detectable harmful effects in laboratory animals or in normotensive patients. It might be especially attractive for targeting of drugs/genes for treatment of pulmonary hypertension. In this disease, local overexpression of ACE (i.e., target for anti-ACE MAb) in distal, small pulmonary arteries (39) may play a role in the muscularization of these vessels, one of the earliest and most consistent features of pulmonary hypertension (38). At the present time, MAb 9B9 is one of the potentially most useful carriers for pulmonary targeting.

A second potential carrier studied in this work was MAb 1A29, which recognizes the transmembrane endothelial surface adhesion molecule ICAM-1 (48, 59). Endothelial cells constitutively express ICAM-1, yet cytokines and other inflammatory mediators cause further stimulation of its expression (50). In the normal perfused rat lung, MAb 1A29 accumulates to the same extent as MAb 9B9 (Fig. 3). However, the pulmonary accumulation of MAb 1A29 in vivo is markedly less selective than that of MAb 9B9 (Table 4). A more promiscuous pattern of 125I-anti-ICAM biodistribution correlates with a more uniform distribution of ICAM-1 in the vasculature in different tissues (Fig. 1 and Tables 2 and 3). Significant accumulation of MAb 1A29 in the liver correlates well with its high content in the hepatic vasculature. It is likely that depletion of the circulating pool of anti-ICAM-1 by nonpulmonary targets limits its pulmonary uptake. Our preliminary studies imply that injection of saturating doses of radiolabeled anti-ICAM-1 (e.g., 10 μg in mice) permits pulmonary accumulation of 10–15% ID/g (Murciano JC and Muzykantov VR, unpublished observations). Thus anti-ICAM-1 represents an attractive carrier for vascular immunotargeting. For example, our recent study documented that anti-ICAM-catalase conjugate binds to the pulmonary endothelium and protects it against oxidative insult similarly to anti-ACE-catalase (1). In addition, anti-ICAM-1 may be useful for drug delivery to inflammation-engaged endothelium. Results from Granger’s laboratory (48) indicate that radiolabeled anti-ICAM accumulates more effectively in the lungs of cytokine-challenged animals.

Another surface adhesion molecule tested in this study was PECAM-1, a member of the Ig superfamily expressed constitutively in endothelial cells at the level of several million copies per cell (46). Qualitatively, PECAM-1 immunostaining in the rat tissues was even more pronounced than that of ACE and ICAM-1 (Figs. 1 and 2). Semiquantitative analysis of immunostaining showed that PECAM-1 is homogeneously distributed throughout all larger vessels, yet PECAM staining is detectably weaker in the capillaries (Tables 2 and 3). This observation supports the notion that the term a “pan-endothelial marker” must be used very carefully.

Recent results from several laboratories show that different PECAM antibodies coupled to enzymes or genetic material accumulate in the lungs in vivo in different animal species and can be used as carriers for the delivery of drugs and genes to the pulmonary endothelium (4, 35, 42, 56). In the present study, radiolabeled anti-PECAM displayed a significant, yet rather modest, pulmonary uptake after intravenous injection in rats (Tables 4 and 5) and a relatively poor uptake in the perfused rat lungs (Fig. 3). Low uptake in the isolated rat lung perfused with blood-free buffer implied that the affinity of anti-PECAM is not sufficient for the targeting (total volume of buffer in the perfusion system is 45 ml vs. ~6 ml of plasma in rats). Theoretically, affinity of antigen binding is an individual feature of anti-PECAM clones. To address this issue, we characterized several anti-PECAM clones in terms of binding to immobilized CD31 and pulmonary uptake. Our results indicate that 1) an affinity of anti-PECAM clones binding to human CD31 varies from 5 to 250 nM; 2) these clones display low pulmonary uptake in perfusion and modest uptake in vivo; 3) after conjugation with SA, some anti-PECAM clones (e.g., MAb 62) display very high uptake in the perfused lungs (30–40% ID/g) and in vivo (15% ID/g); and, 4) the magnitude of the effect of SA differs for individual clones, e.g., SA-biotinylated (b) MAb 164.4 conjugate displays an intermediate uptake in perfusion (10% ID/g) and low uptake in vivo (2% ID/g).

We attribute the effect of SA to formation of multivalent complexes possessing a high affinity for binding to PECAM. Variations of the effect of SA with individual anti-PECAM clones may be explained by several reasons. First, the effect of SA on behavior of individual anti-PECAM clones may depend on their initial affinity. However, a very similar effect on pulmonary uptake of b-MAb 62 (Kd 10 nM) and b-Ab Houston (Kd 250 nM) in perfusion indirectly argues against this explanation. We favor an alternative explanation that the size of SA-b-anti-PECAM conjugates may differ for each individual biotinylated MAb clone. Proteins, including Igs, have a different amount of lysine residues suitable for biotinylation. Our recent studies show that various biotinylated MAb clones form complexes with SA that vary in size from 50 nm to 50 μm and that the size of the resulting SA-b-MAb complexes represents an extremely important parameter of the targeting (Wiewrodt R and Muzykantov, unpublished observations). This aspect of immunotargeting is currently under investigation in one of our laboratories (Muzykantov). The present results indicate that formation of anti-PECAM-SA conjugates dramatically facilitates pulmonary targeting of anti-PECAM. It is conceivable to postulate that affinity of binding of multivalent SA-anti-PECAM to CD31 is higher than that of bivalent antibody, albeit at the present time we cannot exclude potential contribution of facilitated internalization. A direct comparison of the Kd for anti-PECAM and SA-anti-PECAM to CD31 represents a formidable
task because interpretation of the results of either classical (e.g., Scatchard linearization) or modern (e.g., BioCore plasmon resonance) analyses depends on a ligand size. However, chemical modification of anti-PECAM with cross-linkers produces multivalent high-affinity, internalizable anti-PECAM complexes useful as carriers for vascular immunotargeting of enzymes and genes to the pulmonary vasculature in intact animals (4, 35, 42).

CD90.1 (Thy-1.1) antigen is a cell-surface glycoprotein initially identified as a cell differentiation marker expressed in the thymus, lymphoid tissues, hemopoietic stem cells, and neurons (6, 24). Our data show that anti-CD90.1 MAbs accumulate in the spleen (18% ID/g tissue) after systemic injection. This result correlates well with the high level of Thy-1.1 antigen in this organ, likely because of a high concentration of Thy-1.1-expressing blood cells and their precursors (Fig. 1). Effective splenic targeting of anti-Thy-1.1 corroborates with the literature indicating that Thy-1.1 antibodies could be used for delivery of liposomes to lymphoid tissues (13, 27). However, endothelial targeting and pulmonary uptake of anti-Thy-1.1 have not been addressed in the literature; to our knowledge, this paper presents the first evidence of pulmonary accumulation of anti-Thy-1.1. The nature and potential applicability of anti-Thy-1.1 uptake in the lungs (so explicitly manifested in the perfused organ) is worthy of further in-depth investigation. The images of tissue immunostainings (Figs. 1 and 2) show that anti-Thy-1.1 binds to endothelial cells. This result corroborates a recent observation (34) and provides a plausible explanation for the pulmonary accumulation of anti-Thy-1.1. However, pulmonary vasculature is known to contain a significant amount of resident Thy-1.1-positive white blood cells (15). Therefore, both binding to endothelium and to resident white blood cells may contribute to the lung targeting. The applicability of antibodies to Thy-1.1 for vascular immunotargeting must be addressed carefully, especially in the light of potential side effects on Thy-1.1 on the lymphoid and renal cells (45).

Mouse anti-rat endothelial MAb MRC OX-43 accumulates in the perfused rat lungs (Fig. 3). This indicates that the luminal surface of vascular endothelium expresses the antigen recognized by MRC OX-43 and that the affinity of the antibody is sufficient for binding. This MAb recognizes the endothelium of arteries, veins, and capillaries (except brain capillaries). In spleen and in the other organs, significant numbers of interstitial cells are labeled (Figs. 1D and 2E; also see Ref. 54). After in vivo injection, this MAb shows no detectable pulmonary uptake but accumulates in the spleen (7% ID/g tissue) and in the heart (2% ID/g), findings that correlate with immunohistochemical data. Although cardiac accumulation (a rare feature for carrier MAbs) is of interest, the lack of significant pulmonary targeting, as well as the unknown nature of the antigen and its function, limits speculation on potential applicability of MAb MRC OX-43.

In summary, our results support the paradigm that antigen accessibility to the bloodstream and antibody affinity represent more important determinants for the vascular immunotargeting than the total level of an antigen expression. This paradigm is shown in the perfused rat lung by a poor uptake of 125I-anti-PECAM (despite extensive immunostaining) and high uptake of 125I-anti-Thy-1.1 (despite relatively modest immunostaining). PECAM-1 is clearly localized in the vascular endothelium in the lung. Therefore, even a well-expressed endothelial antigen may provide a relatively poor targeting if an epitope is not accessible to the circulating antibody or if the antibody affinity does not suffice. In this context, means for augmentation of carrier affinity (such as SA modification) are of significant interest. In general, the tissue selectivity of MAb uptake correlates with that of an antigen presentation in the capillaries. It can be explained by the fact that capillaries represent the most extended endothelial target surface area in many organs. This paradigm is shown by the selective accumulation of anti-ACE MAb in the lungs, in good agreement with a high selectivity of ACE expression in the pulmonary capillaries. In fact, only the ACE antibody demonstrates a tissue-selective pulmonary uptake among the tested antibodies.

Results of this and other recent studies indicate that anti-ACE, anti-ICAM-1, and anti-PECAM-1 may serve as carriers for vascular immunotargeting, including delivery of drugs, enzymes, and genes to the pulmonary endothelium. Lung perfusion may permit even more effective and safe pulmonary targeting, potentially applicable in lung transplantation and cardiopulmonary bypass.

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