Immunoliposome Targeting to Mesangial Cells: A Promising Strategy for Specific Drug Delivery to the Kidney

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Mesangial cell–mediated nephropathies are a frequent cause of ESRD. Specific drug delivery to mesangial cells might be more effective and better tolerated than existing systemic treatments. Rat mesangial cells are characterized by Thy1.1 antigen expression. Therefore, OX7-coupled immunoliposomes (OX7-IL) were prepared by coupling liposomes with F\textsubscript{ab\textprime} fragments of OX7 mAb directed against Thy1.1 antigen. As the glomerular endothelium is fenestrated and no basement membrane separates glomerular capillaries from the mesangium, mesangial cells represent a particularly suitable target for drug delivery by OX7-IL. Therefore, the targeting efficacy of OX7-IL to mesangial cells was investigated. Specific targeting in \textit{vivo} was obtained, and intravenous injection of OX7-IL to rats showed a specific targeting of all mesangial cells in both kidneys. OX7-IL showed marked accumulation in the cytoplasm of rat mesangial cells, both in \textit{vivo} and \textit{in vitro}. This renal targeting was blocked when free OX7 F\textsubscript{ab}\textsubscript{y}\textsubscript{2} fragments were co-administered with OX7-IL. Rats that were given a single intravenous injection of low-dose doxorubicin encapsulated in OX7-IL showed extensive glomerular damage, whereas other parts of the kidney and other organs were spared. Free doxorubicin and the liposomal formulation of this agent had no effect. Thus, immunoliposomes are a very promising delivery system for the treatment of kidney diseases.


M esangial cells play a central role in maintaining the structure and function of the renal glomerulus. By virtue of their contractile capacities, they also participate in regulating the surface area of the glomerular filtration barrier. Mesangial cell disorders are an important factor in many glomerulopathies, and increased mesangial cell proliferation occurs in a broad range of kidney diseases.

The number of patients with ESRD is on the increase (1,2). Renal transplantation is a potentially life-saving treatment but is costly and carries a high rate of morbidity and mortality (2,3). In the Western world, it is estimated that between 100 and 350 new patients per million inhabitants require treatment for ESRD each year (1). The estimated prevalence of glomerulonephritis among these patients is approximately 15% (1,4). Chronic glomerulonephritis is especially difficult to treat, and novel therapeutic approaches are urgently needed. IgA nephropathy, characterized by excess mesangial cell proliferation and matrix accumulation, is considered to be the most common form of glomerulonephritis worldwide (5–7). IgA nephropathy leads to ESRD in up to 40% of cases within 20 yr of onset (6,7). Current treatments that are designed to slow disease progression mainly comprise immunosuppressive drugs such as corticosteroids and cyclophosphamide (5–9), which have potentially severe systemic adverse effects such as infection and malignancy. Mesangial cell disorders may also contribute to other glomerulopathies such as lupus nephritis and early diabetic nephropathy. Thus, selective drug delivery to glomerular mesangial cells might considerably improve the therapeutic outcome of IgA nephropathy and other glomerulopathies.

Liposomes and immunoliposomes, composed of a phospholipid membrane bilayer, are already used to deliver a wide range of drugs (10,11), and many of the obstacles to liposomal drug delivery have been overcome (12–14). Immunoliposomes have been used mostly for antitumor drug delivery (14,15), but an increasing number of applications that target nonmalignant tissues are emerging (11,16,17).

Mesangial cells represent a particularly suitable target for this strategy, as the glomerular endothelium is fenestrated and there is no basement membrane between the glomerular capillaries and mesangium. Mesangial cells in the rat kidney are characterized by their surface expression of the Thy1.1 antigen (18), which can be used for selective targeting. A specific mAb to rat Thy 1.1. antigen, OX7, is widely available.

Here we used OX7-coupled immunoliposomes (OX7-IL) to target renal glomerular mesangial cells. \textit{In vitro}, specific binding and accumulation in cytoplasm of OX7-IL in mesangial cells was examined. We explored whether renal glomerular mesangial cells could be targeted by OX7-IL \textit{in vivo} and, if so, whether OX7-IL could be used to target doxorubicin to the rat kidney \textit{in vivo}, thereby providing a previously unexplored specific method of treating glomerulonephritis.

Materials and Methods

\textbf{Materials}

3 Sn-phosphatidylcholine (PC), 5(6)-carboxyfluorescein-N-succinimidyld-ester, and doxorubicin hydrochloride were purchased from Fluka

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at least three times.

Cold PBS and analyzed with a FACScan apparatus. Data were analyzed above, then washed twice with cold PBS and fixed with 4% paraformaldehyde. We used three samples per point, and the experiments were performed at least three times.

**Animals**

Male Wistar rats that weighed 200 to 250 g were used for all *in vivo* experiments. They were handled in keeping with our institutional guidelines.

**Preparation of Liposomes and OX7-IL**

Hybridoma cells that produce OX7 mAb were provided by J. Floege (Aachen, Germany). We produced Fab′ fragments and coupled them to DSPE-PEG-Mal as described previously (19). Microparticles were prepared by the detergent depletion method, as described previously (19), from a mixture of PC and DSPE-PEG (94:6 molar ratio). Fab′ fragments (0.1 mg/mlm lipid) were added to produce immunoliposomes.

**FITC-Labeled Liposomes and OX7-IL**

These particles were prepared by the same method, except that carboxyfluorescein-N-succinimidyl ester coupled to PE was built into the lipid bilayer, with the following molar ratio: PC:PE:DSPE-PEG 79:15:6.

**14C-Labeled Liposomes and OX7-IL**

These particles were prepared using the same method, except that 14C-labeled L-3-phosphatidylinositol, 1,2-di(14)oleyl (Amersham Biosciences, Piscataway, NJ) was inserted into the lipid bilayer, with the following molar ratio: PC:14CPC:DSPE-PEG 47:0.08:3.

Doxorubicin Encapsulation in Liposomes and OX7-IL.

Doxorubicin was loaded into liposomes and immunoliposomes via a proton gradient generated by particle-entrapped ammonium sulfate as described previously (19). The doxorubicin concentration, determined by measuring absorbance at 480 nm, was 88 μg/ml in both liposomes and OX7-IL.

**Binding Assay**

For characterizing the binding specificity of OX7-IL, FITC-labeled liposomes and OX7-IL were incubated with the cell lines RMC (Thy1.1+) and K635 (Thy1.1−).

**Fluorometry.**

Cells were cultured in 75-cm² flasks. Confluent cultures were trypsinized, and one million cells were incubated with FITC-liposomes and FITC-OX7-IL in full completed medium at 4°C for 15 min. The cells then were washed twice with cold PBS and lysed with 1 ml of 0.1% Triton-0.1 N NaOH solution. The fluorescence of cell lysates was measured with a Perkin Elmer 3000 fluorometer (excitation 488; emission 520; Perkin Elmer; Boston, MA), and the protein concentration was determined with the Pierce kit (Rockford, IL) on a BioRad microplate reader (Hercules, CA). We used three samples per point, and the experiments were performed at least three times.

**Flow Cytometry.**

Cells were cultured and assayed as described above, then washed twice with cold PBS and fixed with 4% paraformaldehyde in PBS. After centrifugation, the cells were resuspended in cold PBS and analyzed with a FACScan apparatus. Data were analyzed with Cytomation Summit software. The experiments were performed at least three times.
single dose of FITC-immunoliposomes corresponding to 6.6 mmol of total lipid. Rats were anesthetized after 2 h, as described above. Tissues were fixed in situ by intracardiac perfusion of 10% formalin in phosphate buffer. The kidneys then were placed in 10% formalin for 48 h, then dried and placed in 15 and 30% sucrose solution for 1 and 12 h, respectively. The tissues then were embedded in Tissue Tech and frozen on dry ice. Five-micron sections were prepared and incubated respectively. The tissues then were embedded in Tissue Tech and then dried and placed in 15 and 30% sucrose solution for 1 and 12 h, phosphate buffer. The kidneys then were placed in 10% formalin for 48 h, then embedded in paraffin. Sections of each kidney 1 μm thick were stained with hematoxylin and eosin. The pharmacologic effect of doxorubicin on the glomeruli was quantified by the evaluation of the glomerular cell content and by the calculation of the percentage of the damaged glomerular surface area. These investigations were performed by a single investigator with Image J Software in a blinded manner using light microscopy of hematoxylin and eosin-stained kidney sections of 1 μm thickness. Histomorphologic damage analyses of glomeruli were performed by light microscopy with periodic acid-Schiff-stained kidney sections of 1 μm thickness.

Statistical Analyses
Results represented in Figures 1, 2, and 3 are expressed as mean ± SD, and comparison of means was done by t test. Results depicted in Figure 8A (see Results) are expressed as mean ± SEM, and comparison of means was done by t test. Comparison of results in Figure 8B was done by Khideux test.

Results

Microparticle Synthesis and Characteristics
Liposomes and OX7-IL were prepared by the detergent depletion method. Mean liposome and immunoliposome size, measured with a Coulter N4 Plus (Coulter, Miami, FL), was 130 and 170 nm, respectively.

In Vitro Binding Assay
A fluorescence binding assay (Figure 1A) was performed at 4°C to prevent cellular uptake. Cells and microparticles were incubated in culture medium that contained 10% FCS to prevent nonspecific binding. Liposomes and OX7-IL both showed little affinity for the Thy1.1-negative cell line K635. In contrast, OX7-IL showed sixfold higher affinity than liposomes for the Thy1.1-positive cell line RMC. These results were confirmed by FACS analysis (Figure 1, B and C).

Cellular Uptake In Vitro
The capacity of both microparticles to be taken up by RMC that were cultured in complete medium at 37°C (Figure 2A)

Figure 1. In vitro OX7-coupled immunoliposomes (OX7-IL) cell binding. Liposomes and OX7-IL were incubated at 4°C for 15 min with rat mesangial cells (RMC; Thy1.1+) and K635 cells (Thy1.1–). The fluorescence of cell lysates was quantified with a fluorometer (■, liposomes; □, OX7-IL; A). Fluorescence of whole cells was analyzed by flow cytometry: K635 cells (B) and RMC (C) (■, control; solid line, liposomes; dotted line, OX7-IL). **p < 0.0001 versus OX7-IL on RMC.

was evaluated. After 6 h of incubation, the OX7-IL signal was 6.4-fold higher than the liposome signal, and the slope of the uptake curve was much steeper. Confocal microscopy confirmed the more efficient internalization of OX7-IL. Cytoplasmic accumulation of both microparticles was higher at 37°C (Figure 2, C and E) than at 4°C (Figure 2, B and D). Furthermore, cytoplasmic accumulation at 37°C was much higher with OX7-IL (Figure 2E) than with liposomes (Figure 2C).

Pharmacokinetics and Kidney Targeting

Pharmacokinetics. Liposomes and OX7-IL were clear from the plasma compartment, which occurred in biexponential manner. The half life (T1/2), apparent volume of distribution, blood clearance and area under the curve (AUC0-∞) were calculated by noncompartmental analysis from the data in Figure 3A. OX7-IL had a shorter T1/2 and a smaller AUC0-∞ than liposomes and 10-fold more rapid clearance (Table 1). Co-injection of a 10-fold excess of Fabs1/2 fragments increased the T1/2 and AUC0-∞ of OX7-IL and reduced the blood clearance, all by a factor of approximately 5 (Table 1). This showed that the faster clearance of OX7-IL from the bloodstream was largely mediated by their specific targeting.
Kidney Targeting. OX7-IL accumulation in the kidney in vivo and whether such accumulation was due to Thy1.1 antigen recognition by OX7 Fab’ fragments were examined. Rats received intravenous injections of liposomes, OX7-IL, or OX7-IL plus a 10-fold excess of F(ab’/H11032)2 fragments. Microparticle affinity was measured as described previously (17). The kidney volume of distribution was 6.4-fold and sixfold higher with OX7-IL than with liposomes and OX7-IL/H11001 F(ab’/H11032)2, respectively. Furthermore, the %ID and PSA were significantly higher with OX7-IL than with liposomes (Figure 3B). More important, no difference in these three parameters was observed between liposomes and OX7-IL/H11001 F(ab’/H11032)2.

Intrarenal Fluorescence. No fluorescence was observed 2 h after an intravenous injection of FITC-labeled liposomes (Figure 4B) or OX7-IL+ F(ab’/H11032)2 (Figure 4D). In contrast, all glomeruli showed very bright green fluorescence after an intravenous injection of FITC-labeled OX7-IL (Figure 4C). These results confirmed the specific targeting of OX7-IL to the kidney.

Extrarenal Fluorescence. Some fluorescence was observed within the macrophage area in the splenic medulla 2 h after an intravenous injection of FITC-labeled liposomes (Figure 5A’) and at a weaker level with FITC-labeled OX7-IL (Figure 5A”). Moreover, liposomes showed a bright green fluorescence in the liver within the Kupfer cell area, whereas OX7-IL (Figure 5B”) showed almost no fluorescence (Figure 5B”). These results confirmed that the fast OX7-IL clearance was mediated by the specific targeting and not by the reticulo-endothelial system.

Confocal Microscopy
The in vivo specificity of OX7-IL for mesangial cells was investigated. After intravenous injection of FITC-labeled OX7-IL, frozen kidney sections were prepared and stained with Texas Red (TxBR)-labeled OX7 to label the mesangium. Nuclei were counterstained in blue with Hoechst 33342 (Figure 6, C)
and I). Using a Leica confocal microscope, the photography of each color was performed individually because of overlaps between light spectra of the three colors. Then, pictures were merged with help of the Image J software for evaluation of co-localization. The red and green signals completely overlapped (Figure 6, A and B). Nuclei of presumed epithelial or endothelial cells were not associated with red or green staining (Figure 6, F and L, pink arrows), further confirming the specific targeting of OX7-IL to mesangial cells.

**Figure 4.** Renal distribution of OX7-IL in vivo. Epifluorescence microscopy of frozen kidney sections 2 h after intravenous injection of PBS (A and A'), FITC-labeled liposomes (B and B'), FITC-labeled OX7-IL (C and C'), and FITC-labeled OX7-IL + OX7 F(\text{ab}'\text{2}) fragments (10-fold excess; D and D'). Magnification, ×100 in A through D; ×400 in A' through D'.

**Figure 5.** OX7-IL uptake by the reticulo-endothelial system. Epifluorescence microscopy of frozen spleen (A and A') and liver (B and B') sections 2 h after intravenous injection of PBS (A and B), FITC-labeled liposomes (A' and B'), and FITC-labeled OX7-IL (A'' and B''). Magnification, ×200.

For investigating the internalization of OX7-IL in vivo, the mesangium was immunolabeled with TxR-labeled OX7 (Figure 6G), followed by a FITC-labeled secondary antibody (Figure 6H). The overlain staining patterns obtained in control rats (Figure 6, J and K) were uniformly yellow, reflecting the complete expected co-localization. In contrast, the merger between OX7-IL-related fluorescence and the red mesangial staining (Figure 6, D and E) is clearly tricolor. The red coloration represents the cell outer membrane, whereas the yellow coloration represents the co-localization of Thy1.1 antigen and OX7-IL. The latter was seen in the cell membrane, the endosomes, and the cytoplasm, where Thy1.1 protein is produced. The green spots within the cytoplasm (Figure 6, D and E, white arrows) represent internalized immunoliposomes.

**Drug Delivery Model**

Free doxorubicin, doxorubicin-loaded liposomes, empty liposomes, or empty immunoliposomes induced no visible his-

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<table>
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<th></th>
<th>$T_{1/2}$ (min)</th>
<th>Systemic Volume of Distribution (ml/kg)</th>
<th>Blood Clearance (ml/min per kg)</th>
<th>$\text{AUC}_{0-\infty}$ (dpm/min per μl)</th>
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<td>Liposomes</td>
<td>210 ± 42</td>
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<tr>
<td>Immunoliposomes</td>
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<td>89 ± 5</td>
<td>2.46 ± 0.30</td>
<td>367 ± 21</td>
</tr>
<tr>
<td>Immunoliposomes + F(\text{ab}'\text{2})</td>
<td>122 ± 28</td>
<td>87 ± 7</td>
<td>0.52 ± 0.20</td>
<td>1855 ± 580</td>
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$^a$OX7-IL, OX7-coupled immunoliposomes; $T_{1/2}$, half-life; $\text{AUC}_{0-\infty}$, area under the curve.
tomorphologic changes within the glomeruli (Figures 7, A through E, and 8B). In contrast, doxorubicin-loaded OX7-IL damaged all of the glomeruli (Figures 7F and 8B). Glomeruli of rats that were treated with free doxorubicin, doxorubicin-loaded liposomes, empty liposomes, or empty immunoliposomes demonstrated identical cell content. In contrast, glomeruli that were exposed to doxorubicin-loaded OX7-IL showed a significantly decreased cellularity (Figure 8A). In addition, rats that were treated with doxorubicin-loaded OX7-IL showed strong and homogenous glomerular alterations: 56.7 and 33.3% of glomeruli had a damaged glomerular area between 25% and 50% and 50% and 75% (Figure 8B), respectively. Periodic acid-Schiff-stained kidney sections showed typical alterations of severe tissue damage (Figure 9). Vacuolization appeared at the outer

Figure 6. Renal co-localization of OX7-IL with a mesangial marker. (A through F) Kidney section 2 h after intravenous injection of FITC-labeled OX7-IL. (G through L) Control section. Immunolabeling of the mesangium (Thy1.1) by OX7-TxR (A and G), FITC OX7-IL fluorescence (B), and the mesangium (OX7) by a FITC-labeled goat anti-mouse secondary antibody (H), Hoechst staining of nuclei (C and I); A and B superimposed (D and E), G and H superimposed (J and K), C and D superimposed (F), and I and J superimposed (L). Note the intracellular accumulation of OX7-IL (white arrows) and the nuclei of intraglomerular nonmesangial cells (pink arrows).
Finally, this vacuolization evolved into a complete destruction of glomerular tissues (Figure 9, C and D, yellow arrows). During all different glomerular destruction stages, nuclei of dying cells can be observed in the vacuolated areas (Figure 9). No toxicity was observed in any of the tubular areas or in other organs (liver, lung, spleen, heart, or brain).

**Discussion**

A number of conclusions can be drawn from these results. First, OX7-IL have high affinity and specificity for RMC, both *in vitro* (Figure 1) and *in vivo* (Figure 6). Second, OX7-IL are avidly internalized by RMC, *in vitro* (Figure 2) and *in vivo* (Figure 6). Third, OX7-IL pass readily from the bloodstream to the mesangium via the fenestrated endothelium and strongly target mesangial cells (Figure 4C) despite very rapid clearance of the microparticles from the bloodstream (Figure 3A). Fourth, intravenously injected OX7-IL targeted the entire mesangial cell population within all of the glomeruli of both kidneys (Figures 4C and 6). Finally, a specific pharmacologic effect was observed on the renal glomeruli after systemic administration of a low dose of doxorubicin-loaded OX7-IL (Figures 7 through 9).

The mean sizes of liposomes and OX7-IL are 130 and 170 nm, respectively, which is small enough to cross the fenestrated endothelium of glomeruli but too large to pass through the extraglomerular tight endothelium and the glomerular basement membrane (10,20). Thus, OX7-IL gain direct access to the mesangium but are not filtered by the glomerulus and thereby excreted or available for internalization by tubular cells. No OX7-IL were detected in extraglomerular regions of the kidney (Figure 4). OX7-IL had a very high binding specificity for RMC *in vitro* (Figure 1), showing sixfold higher affinity than simple liposomes and no significant affinity for Thy1.1-negative K635 cells.

Although liposomes and OX7-IL both had access to the renal mesangium, only OX7-IL were observed, exclusively within glomeruli (Figure 4, B and C). Moreover, OX7-IL had higher %ID and PSA values than liposomes, further confirming their specific affinity for the kidney (Figure 3B). When OX7-IL were co-injected with OX7 F(ab′)2 fragments, their pharmacokinetic behavior (Figure 3B) and intraglomerular fluorescence signal (Figure 4D) became similar to those of simple liposomes, showing that OX7-IL specifically accumulated in renal glomeruli.

OX7 IgG is highly specific for the mesangium in rat glomeruli (21). The observed co-localization of FITC–OX7-IL and TxR OX7 (Figure 6D) showed that mesangial cells were the only targeted cell type in renal glomeruli and, thus, in the entire kidney. The perfect match between FITC–OX7-IL and the mesangium (Figure 6, A and B) demonstrated the capacity of OX7-IL to target all mesangial cells in a given glomerulus and, thus, in the entire body.

Internalization of OX7-IL by mesangial cells, followed by cytoplasmic drug accumulation, was demonstrated by confocal microscopy both *in vitro* (Figure 2E) and *in vivo* (Figure 6). Rapid OX7-IL uptake was observed in cell culture, as shown by the increase in fluorescence (Figure 2A). The mechanisms of internalization have been described extensively in the literature. After cell binding, the immunoliposomes can deliver
drugs into the cytoplasm by cell membrane fusion or by lysosome membrane fusion after endocytosis (14). The two phenomena both probably are responsible for OX7-IL internalization by mesangial cells, but the exact proportion of the contribution by each mechanism is still unknown (22).

We found that OX7-IL uptake was time dependent and that RMC rapidly internalized OX7-IL. Almost all OX7-IL had disappeared from the bloodstream after 90 min. Meanwhile, this short period was sufficient for OX7-IL to bind at RMC surface and to be accumulated into their cytoplasm. OX7-IL clearance was markedly reduced when OX7 F(ab')2 fragments were co-administered in vivo (Figure 3A). Moreover, OX7-IL were less captured by macrophages in the spleen than liposomes. In the liver, liposomes were significantly taken up by Kupffer cells, whereas OX7-IL were almost not observable. These findings confirm that the fast blood clearance of OX7-IL was not a consequence of the reticulo-endothelial system uptake but of the specific binding to the Thy1.1 antigen. In addition, renal targeting was blocked by co-administration of OX7 F(ab')2 fragments (Figure 3B) and also OX7-IL accumulation in mesangial cells (Figure 4D). These findings demonstrated that OX7-IL accumulation into RMC cytoplasm is fast and mediated by the specific targeting.

These results were confirmed by pharmacologic studies. All glomeruli showed strong acute toxicity within 24 h after a single low dose (0.25 mg/kg) of doxorubicin encapsulated in OX7-IL. This confirms a specific accumulation of doxorubicin within mesangial cells followed by cytoplasmic release, because this drug must reach the nucleus to exert toxic effect on cells. By comparison, Yagmurca et al. (23) injected a single dose of free doxorubicin 80 times higher (20 mg/kg) and observed similar but milder acute toxicity after 10 d, associated with marked tubule damage. We observed cell lysis (Figure 9) and severe vacuolization within glomeruli (Figure 9) well compatible with mesangiolysis (24), occasionally even evolving into a complete glomerular destruction (Figure 9, C and D). Indeed, glomerular structure was strongly damaged: 40% of glomeruli demonstrated a damaged glomerular area >50% (Figure 8B). Strong initial mesangial destruction may be responsible for this histomorphologic damage, because the mesangium plays a central role in maintaining glomerular structure. However, podocytes and endothelial cells also may be affected, possibly through a bystander effect via doxorubicin liberated by destroyed mesangial cells. We observed no histomorphologic damage to other organs, including the brain, heart, liver, lung, and spleen, whereas other authors have reported acute cardiac and hepatic toxicity with free doxorubicin (25).

The renal mesangium seems particularly amenable to drug delivery by immunoliposomes, being readily accessible from the bloodstream via the fenestrated endothelium. In contrast, other organs, such as the brain, have a very tight endothelium that microparticles cannot normally cross (10,17). This easy access allowed us to target homogeneously the mesangium and deliver molecules to the entire population of mesangial cells of the organism. This is not possible for other targets, such as solid tumors (13,15). In addition, mesangial cells have a high natural

![Figure 8](page.png)

**Figure 8.** Quantification of glomerular damage after intravenous administration of doxorubicin-loaded OX7-IL into healthy rats. Randomly selected glomeruli (n = 30) of H&E-stained kidney sections were analyzed with Image J software. (A) Number of cells per glomerular area were measured 24 h after intravenous injection of: PBS (■), empty liposomes (Ⅲ), empty OX7-IL (●), free doxorubicin (□), doxorubicin-loaded liposomes (□), and doxorubicin-loaded OX7-IL (■). (B) Evaluation of the percentage of the damaged area within a given glomerulus. ***P < 0.0001 versus the others; a, b, c, d, and e are not significantly different, but f is significantly different from a, b, c, d, and e (χ² < 0.001).
capacity of phagocytosis that, when combined with good accessibility, facilitate the accumulation of encapsulated drugs into the cytoplasm.

Sengoelge et al. (26) described a high expression of the Thy1.1 antigen on the human mesangial cell surface, in conjunction with a multitude of other gene products. Thus, in addition to the Thy1.1 antigen, other antigens may be evaluated for the directed targeting of therapeutic agents to mesangial cells to treat human glomerulonephritis. In this respect, several significant advances in liposome and immunoliposome technology have recently been made (12–14). Certain liposome preparations, such as Doxil (doxorubicin loaded; Ortho Biotech, Bridgewater, NJ) and Ambisome (amphotericin B loaded; Astellas Pharma Inc, Japan), have been commercially available for several years. Immunoliposomes that are loaded with doxorubicin have successfully completed Phase I clinical trials (27). The high specificity of OX7-IL for mesangial cells suggests that a strong local pharmacologic effect could be achieved in the renal glomeruli, apparently without noteworthy systemic adverse effects.

The efficacy of this approach could be tested in the rat model of Thy1.1 nephritis (28,29). A humanized anti-Thy1.1 antibody will be necessary before commencing clinical trials.

Microparticles have been used to deliver a wide range of molecules (10,11), mainly in the context of drug delivery (14,30) and diagnostic purposes (31,32). In particular, cationic immunoliposomes have been used successfully for in vivo delivery of plasmids (33) and RNAi agents (11). Another potential use of OX7-IL is to investigate mesangial cell involvement in the pathophysiology of glomerulonephritis. Recent studies by our group and others have shown a role of vascular endothelial cell–derived growth factor (34), plasminogen activator inhibitor type 1 (35), thrombin (36), and matrix metalloproteinases (28,29).

Other systems that are used to target drugs and genes to the kidney (37), even to the glomeruli, include virosomes (38) and immunovirosomes (39) that contain sendai virus hemagglutinin. However, these microparticles are not specific for mesangial cells (38,39).

After systemic injection, OX7-IL were found exclusively within renal mesangial cells. Only glomeruli were damaged when OX7-IL were loaded with doxorubicin; the proximal and distal tubules, interstitium, and collecting ducts remained unaffected. Virosomes must be injected directly into the renal artery and are commonly used for immunization strategies.

Figure 9. Histomorphologic analysis of glomerular damage after intravenous administration of doxorubicin-loaded OX7-IL into healthy rats. Light microscopy of kidney sections 24 h after intravenous injection. (A) Glomerulus with a small damaged area (<25% of total glomerular area). (B and C) Glomeruli with a moderately damaged area (25≤50% of total glomerular area). (D) Glomerulus with a highly damaged area (>50% of total glomerular area). Magnification, ×1000, periodic acid-Schiff staining. Note nuclei of dying cells (black arrows), vacuolization (red arrows), and complete glomerular destruction (yellow arrows).
because they are highly immunogenic (13,40), creating a major obstacle to repeated use (rapid clearance, risk for anaphylaxis). In contrast, immunoliposomes showed no major toxicity after repeated injections to rats (41) or during Phase I clinical trials (27).

Thus, OX7-IL can be used as a unique functional genomic tool to clarify physiologic and pathologic processes in the kidney. Immunoliposomes also represent a very effective drug delivery system for a more specific treatment of mesangial cell–mediated forms of glomerulopathies. In the future, we can imagine that such an approach may attenuate the growing number of patients who are developing ESRD.

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References