Synthesis and in Vitro Testing of J591 Antibody–Dendrimer Conjugates for Targeted Prostate Cancer Therapy

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Targeted therapeutics using antibodies are an attractive option over conventional cancer chemotherapy due to their potential to deliver a therapeutic specifically to cancer tissue without damaging normal tissue. However, there are known problems with immunoconjugates such as decreased immunoreactivity and poor solubility. Using dendrimers as carriers for these agents has the potential to resolve these issues. We synthesized J591 anti-PSMA (prostate specific membrane antigen) antibody dendrimer conjugates containing fluorophores on the dendrimer. The in vitro studies of these conjugates show that they specifically bind to cells expressing PSMA. Confocal microscopy experiments document the binding and internalization of these conjugates. This research encourages the further study of antibody–dendrimer–drug conjugates for use in targeted therapeutics.

INTRODUCTION

Targeted therapy for cancer offers potential improvements over existing chemotherapy regimens since the drug is preferentially delivered to the cancer tissue. The side effects associated with chemotherapy can be minimized by targeted drug delivery. Moreover, a prodrug attached to a carrier and a targeting agent would further reduce general cytotoxicity as the drug does not become active until it is released in the targeted tissue.

Monoclonal antibodies (mAb) are useful for the targeted delivery of therapeutics (1), imaging agents, and radiotherapy to tumors. For targeted therapy, identifying an antigen that is uniquely expressed or overexpressed on tumor cells is of crucial importance since this is essential to target the delivery of drugs to tumors while sparing normal tissue. One such antigen for prostate cancer is the prostate-specific membrane antigen (PSMA) (2, 3). PSMA is a 100-kDa, type II membrane glycoprotein highly expressed by all prostate cancers as well as by nonprostatic tumor neovasculature and the vascular endothelium of virtually all solid sarcoma and carcinoma tumors (4). Important to note is that it is not expressed on normal vascular endothelium. Several anti-PSMA mAbs that bind the extracellular PSMA domain might be useful in targeting tumor cells that have been developed (5). These antibodies define two distinct, noncompeting epitopes on the extracellular domain of PSMA. They induce and increase the rate of internalization of PSMA. The internalized antibodies accumulate in endosomes (6). This has led to in vitro and in vivo investigations of these antibodies for immunotherapy (7) and radioimmunotherapy (8–11) for which these antibodies are ideally suited.

Dendrimers (12, 13) are synthetic, nanometer-sized macromolecules that can be modified to suit a specific application. Several types of dendrimers are commercially available, among which PAMAM dendrimers are the most extensively studied for biological applications (14, 15). They have a unique architecture based on β-alanine subunits with primary amine groups on the surface that are available for the attachment of several types of biological material (16). Their aqueous solubility and biocompatibility are well suited to carry ligands, fluorochromes, and drugs for targeting, imaging (17), and drug delivery (18–21). Some of the issues associated with immunoconjugates, such as decreased solubility and reduced binding efficiency, can be addressed using dendrimers as carrier molecules attached to antibodies (22). Several groups have studied the conjugation of dendrimers to antibodies for targeting applications (17, 23–25). Antibody–dendrimer conjugates have been used for radiolabeling (26) with minimal loss of immunoreactivity (29). The current work shows that the anti-PSMA antibody J591 (27), when conjugated to a dendrimer containing a fluorochrome, can be used for targeting prostate cancer and has potential as an efficient delivery system for therapeutics and imaging agents.

EXPERIMENTAL PROCEDURES

Materials. The anti-PSMA antibody, J591, which binds with high affinity to the extracellular domain of PSMA, was used in binding and uptake studies (28). Goat anti-mouse IgG-FITC antibody was purchased from Jackson ImmunoResearch Laboratories (West Grove, PA). Generation 5 PAMAM dendrimers were synthesized in our lab by Dr. Douglas Swanson and characterized by 1H and 13C NMR, GPC, and MALDI-TOF mass spectrometry. Fluorescein isothiocyanate (FITC), dithiothreitol (DTT), N-ethylmaleimide, acetic anhydride, triethylamine, methanol, and dimethyl sulfoxide were purchased from Aldrich (St, Louis, MO). 6-Carboxytetramethylrhodamine succinimidyl ester (6-TAMRA SE) was pur-
chased from Molecular Probes (Eugene, OR). The water soluble heterobifunctional cross linkers sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (Sulfo-SMCC) and sulfosuccinimidyl 6-[3’(2-pyridyldithio)propionamido]hexanoate (Sulfo-LC-SPDP) were purchased from Pierce (Rockford, IL). Prepacked sephadex G-25 PD-10 columns were purchased from Amersham Pharmacia Biotech (Piscataway, NJ) and equilibrated with eluting buffer before sample introduction. Pellicon, microcon, and centrifon devices were purchased from Millipore (Bedford, MA). HiPrep Sephacryl S-100, S-200, and S-300 columns were purchased from Amersham Pharmacia Biotech and used in conjunction with the Isco LPLC system with type 11 (254 nm) or type 12 (214 nm) detectors for purification of dendrimer and antibody conjugates. Novex 4–20% trisglycine gels were used for PAGE with trisglycine SDS running buffer. UV measurements were carried out on a Perkin-Elmer Lambda 20 spectrometer. NMR measurements were carried out on a Bruker 500 MHz spectrometer. The PC-3 and LNCaP.FGC cell lines were purchased from American Type Cell Collection (ATCC, Manassas, VA) and grown in RPMI medium supplemented with penicillin (100 units/mL), streptomycin (100 µg/mL), 50 mML-glutamine, 2 mM sodium bicarbonate, 1× non-essential amino acids (100× stock), 100 µg/mL fungizone, and 10% heat-inactivated FBS as the monolayer at 37°C and 5% CO₂.

**Cell Staining Assay.** Approximately 80% confluent cells were washed once with PBS, trypsinized, washed again, and resuspended in 0.1% bovine serum albumin in PBS (washing buffer). One million cells in 100 µL of washing buffer were incubated on ice either with the J591 antibody or with the isotype control antibody at a concentration of 1 µg/mL for 30 min and then washed twice in washing buffer and stained with anti-mouse IgG-FITC antibody for 30 min. In some experiments the cells were incubated with conjugates 12, 13 (see Scheme 3) or respective control nanodevices either on ice or at 37°C for 30 min. After incubation, the cells were washed, resuspended in washing buffer, and analyzed using flow cytometry.

**Flow Cytometry Analysis.** Single cell suspensions of cells incubated with fluorescent compounds were washed with washing buffer. Samples were run on a Coulter EPICS-XL MCL Beckman-Coulter flow cytometer, and data were analyzed using Expo32 software (Beckman-Coulter, Miami, FL).

**Microscopy Analysis.** To determine the bleaching rate of FITC and 6-TAMRA dendrimer conjugates, 1 µL of 1 M solutions of each of the dendrimer conjugates was placed on a slide coated with gelatin and dried. Appropriate filter and lasers were used for imaging. For the FITC conjugate, a 488 nm 60% neutral density filter with a 500–530 band-pass emission filter was used, and for the 6-TAMRA conjugate, a 543 nm 0% neutral density filter with a 650–615 nm band-pass emission filter was used. The gain was set to within 10% of each other (686–700), and the laser intensity was adjusted with the neutral density filter to saturate the detector. Two line scans from the center to the edge of the sample drop were imaged and averaged. This was repeated 999 times.

Three-dimensional imaging was done with a Zeiss 510 Meta LSCM. A Zeiss 40× Plan apo 1.2 NA was used at 4× digital zoom. Both fluorescence and differential interference contrast images were acquired simultaneously using a 543 nm HeNe laser. An image stack was acquired using confocal fluorescence with a 1 airy unit pinhole with appropriate laser and filters for 6-TAMRA (543 nm laser, 560 nm long pass filter). The image stack size, resolution, and sampling rate settings assured that an individual cell could be rendered in 3D with enough detail to discern internalization of the nanodevice. There were 31 optical sections in a 512 × 512 pixel image stack. Each optical section was 1.5 µm thick. The image stacks were imported into Velocity (Improvision, Inc.) for deconvolution and 3D reconstruction. The deconvolution used an iterative maximum entropy algorithm and a theoretical confocal point spread function run to achieve 85% confidence level. Three-dimensional rendering enabled us to select, measure, and identify individual endocytosed vesicles.

**Synthesis of Dendrimer Conjugates.** To a methanolic solution of generation 5 PAMAM dendrimer 1 (1.85 g; 72 µmol) (Scheme 1) under nitrogen, triethylamine (0.56 g; 5.5 mmol), and acetic anhydride (0.552 g; 5.4 mmol) were added dropwise while stirring (20). Stirring continued at an ambient temperature for 16 h. Methanol was removed in vacuo, and the residue was ultrafiltered using a Pellicon Device (10K MWCO), washing initially with a pH 8.0 PBS buffer and then for several recirculations with deionized water. The partially acetylated

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* Reagents and conditions: (a) Acetic anhydride, Et₃N, methanol, rt, 16 h; (b) Sulfo-LC-SPDP, PBS, rt, 3 h; (c) FITC or 6-TAMRA SE, DMSO; (d) DTT, PBS–EDTA.

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**Scheme 1. Synthesis of Dendrimer Conjugates by Terminal Group Modifications on Generation 5 PAMAM Dendrimer**

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**Figure:**

![Graphical representation of Scheme 1](image)
and immediately reacted with dendrimer thiolates

dendrimer conjugate 2 was lyophilized; 1.5 g was recovered. To a solution of conjugate 2 (0.2 g; 7 μmol) in PBS (20 mL), sulfo-LC-SPDP (21 mg; 40 μmol) was added. The unconjugated reagents and byproduct were separated after 3 h by ultracentrifugation using a 10K MWCO Pelicon device, washing with PBS and then with DI water. The conjugate was lyophilized to give 115 mg of disulfide conjugate 3 as an off-white powder.

To the dendrimer disulfide conjugate 3 (45 mg; 1.5 μmol) in DMSO (5 mL), a solution of FITC (3.4 mg; 8.7 μmol) in DMSO (0.5 mL) was slowly added while stirring over a period of 30 min in darkness under nitrogen at ambient temperature. Stirring continued for 16 h. The reaction mixture was cooled on an ice bath, water was added, and the solution was dialyzed in a 15K MWCO regenerated cellulose bag in the dark. The initial buffer exchanges were with PBS and later with deionized water. The resultant purified dendrimer–FITC–disulfide conjugate was lyophilized to yield 35 mg of conjugate 4. Similarly, 6-TAMRA succinimidyl ester (5 mg; 9.4 μmol) in DMSO (0.5 mL) was slowly added to dendrimer conjugate 3 (41 mg; 1.4 μmol) in DMSO (5 mL) at room temperature and stirred in the dark under nitrogen for 16 h. The reaction mixture was then dialyzed in a 15 K MWCO regenerated cellulose membrane. The initial red permeate fractions releasing free 6-TAMRA faded, and the dialysis continued until the permeate fractions were colorless. After dialyzing further in deionized water, the retentate was lyophilized to give 25 mg of dendrimer–6-TAMRA–disulfide conjugate 5 as an amorphous red powder.

The disulfide bonds on dendrimer conjugates 4 and 5 (2 mg each; 64 nmol) were reduced by using 10 mM DTT in degassed PBS-EDTA buffer (0.5 mL) at RT under argon to provide the corresponding dendrimer–thiol conjugates 6 and 7. These conjugates were purified by gel filtration, eluting with PBS-EDTA buffer under nitrogen on a PD-10 column to remove excess reagents and byproducts, and were immediately used for subsequent antibody conjugation.

Dendrimer conjugates without antibody, for in vitro control experiments, were prepared by treating G5 PAMAM dendrimer 1 (100 mg; 4 μmol) with a 6-fold molar excess of FITC (9 mg; 24 μmol) or 6-TAMRA (12.6 mg; 24 μmol) in DMSO (10 mL) under an inert atmosphere for 2 h under an inert atmosphere. Excess thiol groups on the dendrimer were then quenched using N-ethylmaleimide to minimize dimer formation. The products were then concentrated by centrifugal ultrafiltration using YM100 microcon devices to remove excess dendrimer and other reagents. They were further purified on a S-200 Sephacryl column to give conjugates 12 and 13.

RESULTS AND DISCUSSION

Dendrimer Conjugate Characterization. The dendrimer terminal group modification is shown in Scheme 1. Amineterminated PAMAM dendrimers can nonspecifically bind to cells due to the positive surface charge. To improve targeting efficacy and decrease nonspecific interactions, the generation 5 PAMAM dendrimer 1 was partially acetylated. The use of 80 times molar excess of acetic anhydride leaves some amines unreacted on the dendrimer for further conjugation. Acetylation also decreases the intermolecular interactions, minimizing aggregate formation and solubility problems. Initially using pH 7.4 PBS, for ultrafiltration, removes loosely bound side products that would otherwise be retained if ultrafiltered only against water. The purity of the conjugate 2 and the extent of acetylation can be measured (30) and monitored by 1H NMR, which shows a distinct signal for the terminal NHCOCH3 protons of the dendrimer at δ 1.85. If acetic acid contamination is detected upfield at δ 1.80, it can be removed by repeated dialysis in PBS and water.

To introduce a disulfide group, the water-soluble heterobifunctional cross-linking agent sulfo-SPPD was conjugated to the partially acetylated dendrimer 2 to provide a protected thiol in the form of a disulfide, which is later cleaved with DTT. The extent of disulfide modification can be monitored by UV spectroscopy, using the pyridine-2-thione assay (Figure 1) by adding DTT to a measured quantity of dendrimer and recording the absorbance of the released 2-thiopyridine at 343 nm. On the basis of this measurement, it was calculated that there are an average of two disulfide groups per dendrimer in conjugate 3 in agreement with values obtained by 1H NMR by comparing the integration values of the

Scheme 2. Dendrimer Labeled with FITC or 6-TAMRA for Control Experiments

- Reagents and conditions: (a) DMSO, FITC or 6-TAMRA SE; (b) acetic anhydride, Et3N, PBS–DMSO, rt, 12 h.

Antibody Conjugation. Modification of J591 anti-PSMA mAb (Scheme 3). The J591 antibody (10) was provided in 5 mg/mL concentration in PBS buffer and used without dilution. A thiol reactive maleimide group was introduced on the antibody by reacting the J591 mAb with sulfo-SMCC in PBS at ambient temperature for 2 h to give conjugate 11 using standard protocols (29). The excess reagent was removed by gel filtration on a Sephadex G-25 column. This maleimide-linked antibody conjugate 11 was then concentrated on a microcon YM50 and immediately reacted with dendrimer thioldes 6 or 7 in degassed PBS-EDTA buffer at an ambient temper-
heteroaromatic signals of the pyridine and the aliphatic signals of the dendrimer.

Imaging of cells by flow cytometry or confocal microscopy requires a detectable fluorescent probe. We have investigated the use of different fluorescent probes on the dendrimer for this purpose. Remarkably, up to 20 times molar excess of these probes, which have aromatic groups, can be conjugated to the dendrimer while still having the conjugate remain soluble in aqueous buffers. To retain complete solubility but still provide an imaging source for these studies, the amount of FITC labeling was decreased to six per dendrimer. The UV spectrum of conjugate 4 shows absorption for the fluorescein at $\lambda_{\text{max}}$ 498 nm. The $^1$H NMR of the conjugate shows overlapping signals in the aromatic region for both the fluorescein and the pyridine ring from the disulfide linker apart from the expected aliphatic signals for the dendrimer. The fluorescein label is ideally suited for flow cytometry experiments for in vitro studies; it, however, photobleaches under confocal microscopy conditions. For the confocal microscopy experiments, we tested 6-TAMRA, a dye with a higher quantum yield and with minimal photobleaching compared to FITC.

We tested the rate of photobleaching in dendrimer conjugates with FITC and 6-TAMRA (Figure 2). A rapid decrease of 50% fluorescence in dendrimer conjugated to FITC was observed after 25 scans. The fluorescence decreased by only 10% for dendrimer–6-TAMRA conjugate after 25 scans.

Figure 1. Pyridine-2-thione assay. The increase in absorbance at 343 nm for the conjugate is due to the release of 2-thiopyridine.

Figure 2. Comparison of photobleaching of FITC and 6-TAMRA labeled dendrimer conjugates (9a and 9b) in the confocal microscope. A rapid decrease of 50% fluorescence in dendrimer conjugated to FITC was observed after 25 scans. The fluorescence decreased by only 10% for dendrimer–6-TAMRA conjugate after 25 scans.

Reagents and conditions: (a) PBS, pH 7.4, rt, 2 h; (b) conjugate 6 or 7, PBS-EDTA, pH 7.4, rt, 2 h, $N$-ethylmaleimide.

**Scheme 3. Antibody–Dendrimer Conjugation**

![Scheme 3](image)

**Figure 3.** Antibody–Dendrimer Conjugates Bioconjugate Chem., Vol. 15, No. 6, 2004 1177
slight excess resulted in a stable conjugate that is water soluble. The reduction of disulfide bonds on dendrimer conjugates 4 and 5 was carried out by using DTT. The resultant dendrimer thiols 6 and 7 can undergo oxidation and hence the reactions were carried out and purified carefully under an inert atmosphere in degassed PBS-EDTA buffer.

For in vitro control experiments, G5 PAMAM dendrimer was labeled with FITC and 6-TAMRA. The 1H NMR of the purified conjugates 8a and 8b showed additional signals in the aromatic region between δ 6–8 ppm. The UV–vis spectrum of 8a had absorption at λ \text{max} 498 nm for the conjugate. The conjugates 8a and 8b were then completely acetylated with excess acetic anhydride in the presence of triethylamine at pH 8 to give conjugates 9a and 9b. An additional peak at δ 1.8 for the CH₃ of the terminal acetyl group and the merging of methylene proton signals α to the NH₂ in the starting material confirms the complete acetylation of the terminal amines.

A thiol reactive maleimide group was introduced on the antibody with water soluble sulfo-SMCC using standard protocols and the resultant conjugate was purified by gel filtration. To minimize the free antibody, 5-fold molar excess of dendrimer conjugates was used in the antibody–dendrimer conjugation; the unreacted thiols were quenched with N-ethylmaleimide to minimize dimer formation due to cross-linking of dendrimer to antibodies. Free unreacted dendrimer was removed initially by filtration with a 100K MWCO microcon. Further purification on a S-200 Sephacryl column removed any residual free dendrimer. The resultant conjugates 12 and 13 were analyzed using PAGE, which shows a band above the antibody band that confirms the conjugation and an absence of a band for the free dendrimer. The fluores-

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**Figure 3.** PC-3 (a) and LNCaP.FGC (b) cells were incubated either with isotype control or J591 antibodies (1 µg/mL), for 30 min. on ice, and then stained with anti-mouse IgG-FITC antibody, and green fluorescence was analyzed on FCM.

**Figure 4.** PC-3 and LNCaP.FGC cells were stained with increasing concentrations of J591-G5-FITC conjugate (12) (a, top panel), G5-FITC (9a) (b, middle panel) or 150 nM of irrelevant 60BCA-G5-FITC antibody–dendrimer conjugate (c, bottom panel) and analyzed on FCM.

**Figure 5.** Kinetics of binding J591-G5-FITC dendrimer to LNCaP.FGC cells. The cells were incubated with a 50 nM concentration of 12 or control G5-FITC dendrimer (9a) for the indicated periods of time at rt. After incubation, cells were washed and the mean channel fluorescence of stained cells was determined by FCM.
nescence of the conjugates gives an indirect proof of conjugation since the fluorescent probe was conjugated to the dendrimer.

Targeting PSMA-Positive Prostate Cancer Cells with Monoclonal Antibody. To confirm the specificity of the J591 antibody toward the extracellular domain of PSMA, live PSMA-positive LNCaP.FGC cells and PSMA-negative PC-3 cells were incubated with J591 antibody and stained with anti IgG-FITC. The J591 antibody shows a great specificity against PSMA antigen. More than 97% of LNCaP.FGC cells were positively stained with J591 mAb (Figure 3b) compared to a nonspecific stain with the isotype antibody. The PC-3 cells showed (Figure 3a) only background-level staining with both antibodies (J591 and isotype) and no specificity. This confirms that the J591 antibody specifically binds to the LNCaP.FGC cells, and the binding is highly specific (27).

Targeting PSMA-Positive Prostate Cancer Cells Using Monoclonal Antibody–Dendrimer Conjugates. Dendrimer conjugation to the antibody can alter the binding characteristics of the antibody since it has the potential to block the binding sites. To ascertain whether the targeting efficacy and specificity of the antibody–dendrimer conjugate toward PSMA is maintained, three types of conjugates were tested, conjugates 9, 12, and another antibody dendrimer conjugate, 60BCAG5-FITC, as a control (a dendrimer conjugated to an irrelevant antibody). At a 3 nM concentration of 12 (J591-G5-FITC), the LNCaP.FGC reached approximately 25 units of mean channel fluorescence (MCF) (Figure 4a). At 10 times higher concentration, the fluorescence reached an upper plateau at 35 MCF. Staining with a similar concentration (4 nm) of the G5-FITC control dendrimer conjugate 9a (without the antibody) yielded less than 15 MCF (Figure 4b). The MCF never exceeded 20 units even at a 300 nm concentration of conjugate 9a. Both conjugates (9a and 12) have nonspecific staining of PC-3 cells equal to the background (Figure 4) fluorescence. The irrelevant antibody (same class) conjugate (60BCAG5-FITC) did not bind significantly to either cell line (Figure 4c).

We have also examined the kinetics of the binding of conjugate 12 and control dendrimer 9a to LNCaP.FGC cells. The binding of 12 to LNCaP.FGC cells reached a plateau after 30 min. of incubation. The mean fluorescence of cells stained with 12 increased from 14.5 to 68.1 MCF, while the mean fluorescence increase of cells stained with 9a was insignificant (Figure 5). To further prove the specific binding of the dendrimer–antibody conjugate, LNCaP.FGC and PC-3 cells were preincubated with free J591 antibody at 750 ng/mL concentration for 15 min. on ice to block the receptor sites. Dendrimer conjugates 12 and 9a (48 nM) were added, and the cells were incubated for an additional 30 min and then washed and subjected to FCM analysis. Free J591 antibody
inhibited the binding of the conjugate to LNCaP.FGC cells by more than 71%, while nonspecific binding to PSMA-negative cells remained unchanged (Figure 6). This blocking experiment proves that the antibody–dendrimer conjugate retains specific binding to PSMA.

**Internalization of the Dendrimer Conjugates by PSMA-Positive Cells.** Free J591 antibody is readily internalized by LNCaP.FGC cells (6). We examined whether the antibody–dendrimer retains this ready internalization. Initial confocal microscopy studies with the dendrimer conjugate 12 and live cells provided inconclusive results due to the photobleaching of the FITC and background autofluorescence. Changing the fluorescent probe on the dendrimer to 6-TAMRA gave a better signal-to-noise ratio which allowed conclusive measurements. The PC-3 and LNCaP.FGC cells were trypsinized, washed, and incubated with conjugate 13 for 1 h at 37 °C and then washed and analyzed.

LNCaP.FGC cells yielded the highest fluorescence when stained with conjugate 13 (Figure 7). We observed minimal fluorescence in PC-3 cells stained with conjugate 13. To analyze the fluorescence intensity in both cell types quantitatively, we selected 3D-rendered images of representative LNCaP.FGC and PC-3 cells and determined the number of fluorescent voxels per cell. The LNCaP.FGC yielded 12 520 voxels, while PC-3 yielded only 1156. Since both LNCaP and PC-3 are human prostate cell lines grown under identical conditions and they are very similar in size, the order of magnitude difference in the number of fluorescent voxels can be attributed to differences in dendrimer uptake. The confocal images were further analyzed by taking optical sections to determine internalization of the conjugate.

Medial optical scans (Figure 8) clearly show internalization of the antibody–dendrimer conjugate 13 into the PSMA-positive LNCaP.FGC cells. The fluorescence profile shows increased intensity in the cytoplasm but not inside the nucleus.

**CONCLUSION**

Dendrimers are useful as carrier macromolecules for targeting, imaging, and drug delivery agents. Terminal group functionalization of water-soluble PAMAM dendrimers with different aromatic and hydrophobic molecules allows delivery of many types of drugs but may significantly reduce the aqueous solubility of the conjugates. Altering the surface amine functionality to a more neutral acetylated surface reduces the formation of aggregates and retains the water solubility of the conjugates. The acetylated terminal groups also decreased the nonspecific binding to the cells (20). The conjugation of the targeting J591 antibody to surface-modified G5 dendrimers has been achieved. Flow cytometry experiments showed that the antibody–dendrimer conjugate is specifically bound by the PSMA-positive LNCaP.FGC cell line but does not bind to the PC-3 cell line which does not express PSMA. Dendrimers not conjugated to an antibody did not show any significant binding. Confocal microscopy studies also confirmed the binding and internalization of the antibody–dendrimer conjugate, while the dendrimer alone did not show significant binding or uptake into the cells. Studies are under way to synthesize a multifunctional dendrimer conjugate with drug, fluorochrome, and antibody for targeted drug delivery and imaging.

**ACKNOWLEDGMENT**

This project has been funded in whole or in part with Federal Funds from the U.S. Army Medical Research and Material Command, Department of Defense, under grant DAMD17-02-1-0096, National Cancer Institute, National Institutes of Health, under Contract #NO1-CO-97111, and the S.P.O.R.E. National Institutes of Health, under grant 2-P50-CA69568-04.

**LITERATURE CITED**


