In Vitro Targeting of Synthesized Antibody-Conjugated Dendrimer Nanoparticles†

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Received May 20, 2004; Revised Manuscript Received July 26, 2004

This study reports the synthesis and in vitro biological properties of dendrimer−antibody conjugates. The polyamidoamine dendrimer platform was conjugated to fluorescein isothiocyanate as a means to analyze cell binding and internalization. Two different antibodies, 60bca and J591, which bind to CD14 and prostate-specific membrane antigen (PSMA), respectively, were used as model targeting molecules. The binding of the antibody-conjugated dendrimers to antigen-expressing cells was evaluated by flow cytometry, confocal microscopy, and a new two-photon-based optical fiber fluorescence detection system. The conjugates specifically bound to the antigen-expressing cells in a time- and dose-dependent fashion, with affinity similar to that of the free antibody. Confocal microscopic analysis suggested at least some cellular internalization of the dendrimer conjugate. Dendrimer−antibody conjugates are a suitable platform for targeted molecule delivery into antigen-expressing cells.

Introduction

Currently used cancer chemotherapeutics are often inadequate to cure tumors because of the nonselectivity of these drugs, resulting in dose-limiting side effects. The application of drug carrier systems for targeting tumor cells has gained credence as an alternative approach for treating cancer and offers both an increased therapeutic index and decreased drug resistance. In this approach, a carrier molecule is synthesized and coupled to a targeting molecule and a therapeutic, the former to guide the polymer to the tumor site and the latter to induce cell death. The homing of the drug specifically to the cancer cells reduces the toxicity and increases the antitumor efficacy.[1] In addition, polymeric drug conjugates can be formulated to increase drug solubility in aqueous media, further increasing the plasma half-life of a pharmaceutical to decrease the required dose.[2] Several synthetic and natural polymers have been evaluated as carrier molecules for drug delivery in the past decade and have validated the concept of targeting tumor cells.[3−5] However, few of these formulations have achieved clinical approval due to the poor biocompatible properties of these carrier molecules such as insolubility, immunogenecity, and low tissue permeability.[2−5]

An effective targeted drug delivery system requires a platform that is uniform and able to couple multiple components such as a targeting molecule, a drug, a cancer-imaging agent, and a fluorescent probe without degrading the function of the components. Polyamidoamine (PAMAM) dendrimers offer such a carrier system, being uniform, nonimmunogenic, nontoxic, biocompatible molecules with a defined branched chain structure capable of carrying multiple molecular entities that are linked covalently to its surface.[6−9] Dendrimer conjugates have been used experimentally to deliver drugs,[10] DNA,[11] radionuclides,[12] MRI contrast agents,[13] and boron in neutron capture therapy.[14]

Monoclonal antibodies have been widely evaluated as cancer-targeting agents because tumor cells are known to express surface antigens that are specific to the cancer or present in great excess when compared to normal cells. If an overexpressed molecule on a cancer cell is directly involved in growth promotion, an antibody by itself may be useful in inhibiting cell growth in a tumor.[15,16] Antibodies can also be utilized to target a drug into the cell, either by direct conjugation of the drug to the antibody as an "immunoconjugate"[17] or conjugated to a carrier molecule. Polymers such as *N*-(2-hydroxypropyl)methacrylamide (HPMA),[18] poly[*N*5-(2-hydroxyethyl)-l-glutamine] (PHEG),[19] and liposomes[20] have been tested as platforms for antibody targeting. Polymer-based antibody targeting also may overcome some of the limitations of immunoconjugates, such as reduced drug activity, reduced antibody affinity,[21,22] and drug resistance.[23]

We have recently shown the utility of folic acid-conjugated PAMAM dendrimers for in vitro targeting of cultured tumor cells and human xenograft tumors in mice.[24,25] In this report

† Abbreviations: EDTA, ethylenediamine tetraacetic acid; DTT, dithiothrietol; FI (FITC), fluorescein isothiocyanate; G5, generation 5-polyamidoamine (PAMAM) dendrimer; PA, prostate-specific membrane antigen antibody (J591); PBS, phosphate-buffered saline; PSMA, prostate-specific membrane antigen; Sulfo-LC-SPDP, sulfosuccinimidyl-3-(2-pyridyldithio)-propionate; Sulfo-SMCC, sulfosuccinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate; TPOFF, two-photon-based optical fiber fluorescence.

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10.1021/bm049704h CCC: $27.50
Published on Web 09/11/2004

we show the synthesis and in vitro biological properties of two dendrimer−antibody conjugates. We have used G5-PAMAM dendrimer (G5) as a carrier molecule and the antibodies 60bca (60B)[26] and prostate-specific membrane antigen (PSMA) antibody (PA, J591)[27] as targeting agents. Fluorescein isothiocyanate (FITC, FI) was also coupled to the dendrimer as a fluorescence-detecting agent (G5-FI−60B and G5-FI−PA).

HL-60 cells from a human myeloblastic leukemia cell line are known to express the antigen CD14 when induced by 1,25-dihydroxyvitamin D3 to differentiate to monocytes.[28] The PSMA antigen is overexpressed in prostate cancer, and antibodies against PSMA have been clinically tested for targeted therapy.[27] The human prostate cancer cell line LNCaP is known to express PSMA.[29] In this report we show the association of G5-FI−antibody (G5-FI−Ab) in these cell models by use of flow cytometry, confocal microscopy, and a newly developed two-photon-based optical fiber fluorescence (TPOFF) detection system.[25,30]

Materials and Methods

G5-PAMAM dendrimers were prepared at the Center for Biologic Nanotechnology, University of Michigan, and were analyzed extensively by ^{1}H and ^{12}C NMR, matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry, high-performance liquid chromatography (HPLC), gel-permeation chromatography (GPC), and polyacrylamide gel electrophoresis (PAGE). The heterobifunctional cross-linkers sulfosuccinimidyl 3-(2-pyridyldithio)-propionate (sulfo-LC-SPDP) and sulfosuccinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC) were from Pierce (Rockford, IL). Pellicon XL devices (5K and 10K molecular weight cutoff regenerated cellulose) were purchased from Millipore and connected to a pump for ultrafiltration. Pre-packed Sephadex G-25 PD-10 columns and HiPrep Sephacryl S-300 (used in conjunction with fast protein liquid chromatography, FPLC) were purchased from Amersham Pharmacia Biotech (Piscataway, NJ) and equilibrated with degassed eluting buffer before sample introduction. The 1,25-dihydroxyvitamin D3 was from Calbiochem (San Diego, CA). Human promyelocytic leukemia HL-60, Jurkat, LNCaP (LNCaP-FGC), and PC3 cell lines were obtained from American Type Culture Collection (ATCC, Rockville, MD). Trypsin−EDTA, Dulbecco's phosphate-buffered saline (PBS), Iscove's modified Dulbeccos medium (IMDM), and F12 medium were obtained from Gibco/BRL (Gaithersburg, MD). FI-labeled goat anti-mouse anti-IgG was purchased from Jackson Immunological Research Laboratory (West Grove, PA). All other reagents were from Sigma (St. Louis, MO).

Production and Purification of Anti-CD14 mAb (60bca) and Anti-PSMA (PA). Hybridoma cell line 60bca-secreting human blocking anti-CD14 antibody (IgG1; κ light chain) was obtained from ATCC (Rockville, MD). The hybridoma cell line was propagated in IMDM medium + 10% FBS. The cells were collected, rinsed, and resuspended at the concentration of 2.5×10^6 cells/mL in PBS. Six-week-old, nude peristane-pretreated mice were intraperitoneally inoculated with 2 mL of cell suspension (5×10^6 cells/mouse). Two weeks later the ascetic fluid was collected and the monoclonal Ab was purified over a protein G column (Pharmacia, Uppsala, Sweden). PSMA antibody (PA, IgG1), designated J591, that binds to the extracellular domain of PSMA was kindly provided by Dr. Neil H. Bander, Weill Medical College of Cornell University, Ithaca, NY.[27,31]

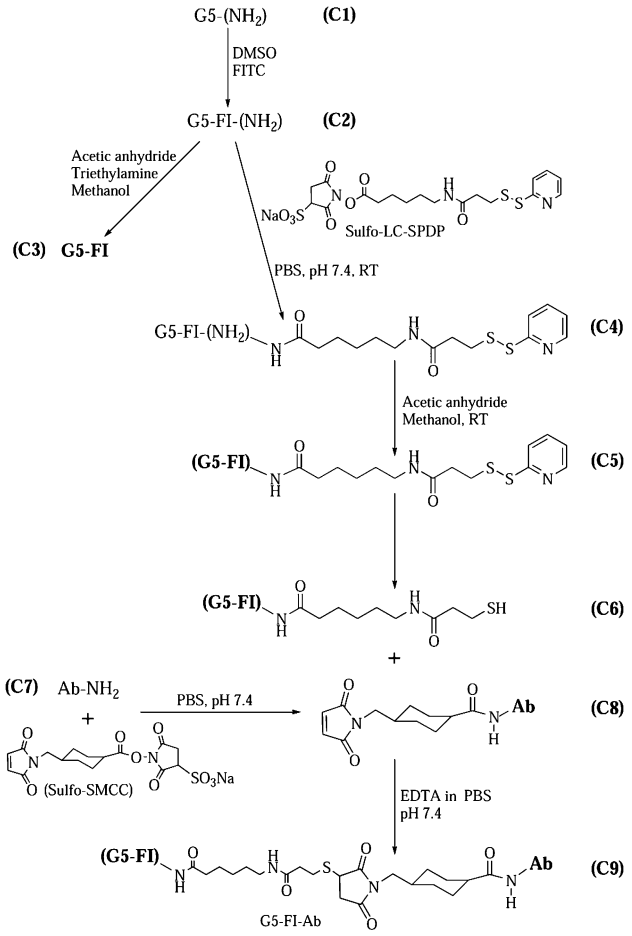

Figure 1. Scheme showing the synthesis of G5-FI−Ab. The details are described in the text.

Preparation and Purification of Antibody−Dendrimer Conjugates. The synthetic procedure is similar to that described previously.[31] A summary of the synthetic steps used in the present study is shown in Figure 1. All the intermediate products are purified either by ultrafiltration through a 10K MW cutoff Pellicon device, by extensive dialysis, or by gel filtration on a PD10 column. G5-PAMAM (2.8 mM; conjugate 1, **C1**, in Figure 1) was treated with a 5-fold molar excess of FI in DMSO to give **C2**. The remaining free amino groups of **C2** were acetylated to produce **C3**, which was used as a control. **C2** was initially functionalized by reacting with a 6-fold molar excess of sulfo-LC-SPDP to obtain **C4**. The terminal amino groups of **C4** were acetylated as given above. The product (**C5**) was reacted with 10 mM DTT and 1 mM EDTA in PBS, pH 7.4, to cleave the disulfide bond and to generate **C6**, which was concentrated and purified.

The antibody (**C7**; 5 mg/mL) was initially coupled to excess sulfo-SMCC in PBS (pH 7.4) by standard protocols to generate **C8**. **C8** was reacted with a 5-fold molar excess

of the synthesized **C6** in PBS for 4 h at ambient temperature to generate **C9**. The excess thiol groups in **C9** were quenched by incubation with ~50-fold molar excess *N*-ethylmaleimide to minimize dimer formation. The final product was ultrafiltered and was further purified by gel filtration on an FPLC S-300 Sephacryl column. Polyacrylamide gel electrophoresis of the purified dendrimer was performed on a 4−20% gradient gel at pH 8.3 under non-reducing conditions and by other standard protocols.

Cell Culture and Treatment. The HL-60 and Jurkat cells were maintained in IMDM medium. To induce the expression of CD14 antigen, 5×10^6 HL60 cells were seeded into a 100-mm dish and exposed to 100 nM 1,25-dihydroxyvitamin D3 for 72 h prior to binding studies. LNCaP cells were cultured in RPMI medium in the presence of 10 mM *N*-(2-hydroxyethyl)piperazine-*N*′-ethanesulfonic acid (HEPES) and 1 mM sodium pyruvate. PSMA-negative PC3 cells were cultured in F12K medium. All the cells were supplemented with 10% fetal calf serum (FCS), 100 units/mL penicillin, and 100 μg/mL streptomycin and were maintained at 37 °C in a humidified atmosphere containing 5% CO_2 and 95% air. For treatment with dendrimer, the HL60 and Jurkat cells and trypsinized LnCaP and PC3 cells were rinsed and resuspended in phosphate-buffered saline containing 0.1% BSA (PBSB). Following incubation with the various concentrations of either the dendrimer conjugates or free antibody, the cells were spun, the incubation medium was removed, and the cells were rinsed twice with PBSB. These incubations were done either at 4 °C, to monitor binding, or at room temperature, to assess both binding and internalization. Free antibodies were added prior to the dendrimer in control experiments to block the binding of the conjugates. For anti-IgG-FITC secondary antibody treatment, dendrimer-treated cells were rinsed and incubated with 10 μg/mL FITC-labeled secondary antibody for 30 min at 4 °C and rinsed as above. The treatment with the dendrimer−antibody conjugate presented in this study did not induce cytotoxicity, as observed by the absence of any morphological changes in the microscopic analysis.

Flow Cytometry and Confocal Microscopy. The FITC fluorescence was quantified on a Becton Dickinson FACScan analyzer. The viable cells were gated, and the mean FL1-fluorescence of 10 000 cells was quantified. Confocal microscopic analysis was performed in cells suspended in PBSB by placing a drop of the cell suspension on a cover slip and allowing the cells to settle down; they were then analyzed in a Carl Zeiss confocal microscope.

Two-Photon Optical Fiber Fluorescence Analysis. The TPOFF analysis was based on two-photon fluorescence measurements through a single-mode optical fiber.[30] A Ti:sapphire laser providing 80-fs pulses at 830 nm with an 80-MHz repetition rate was coupled into the fiber to excite samples in the vicinity of the distal fiber end, and the emitted fluorescence was collected back through the same fiber. The fluorescence was separated from the excitation beam with a dichroic mirror and further filtered with a short-pass filter before being detected by a photon-counting photomultiplier tube. The TPOFF analysis was done for the binding of G5-FI−60B to the HL60 cells. Following G5-FI−60B treatment, 5×10^6 rinsed cells were centrifuged in a microfuge tube, and the supernatant was removed. The fiber was immersed into the cell pellet through a 27-gauge needle, and the emitted fluorescence was recorded every second for 25−30 s in multiple areas of the pellet by physically moving the needle.

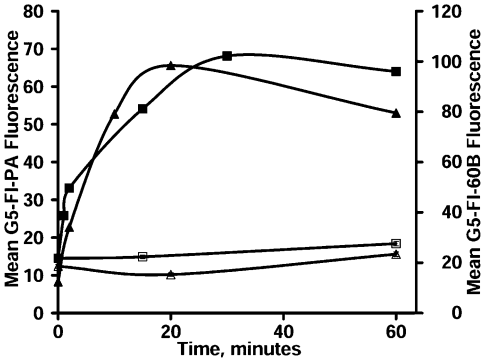

Figure 2. Time course for the binding of G5-FI−Ab to antigen-expressing cells. CD14-expressing HL60 cells (▲) and LnCaP cells (■) were treated respectively with G5-FI−60B (25 nM, 4 °C) or G5-FI−PA (50 nM, room temperature) for different time periods, and the fluorescence of rinsed cells was measured in a flow cytometer. (△) HL60 and (□) LnCaP cells were treated with the control dendrimer G5-FI under similar conditions. The values are the mean ± SD of fluorescence counts for the cell population from a single experiment. Similar results were obtained in a separate experiment.

Statistical significance of differences among groups was analyzed by the Student−Newman−Keuls test, with significance calculated at $P < 0.05$.

Results

The G5-PAMAM dendrimer contains theoretical 128 free terminal primary amino groups, which were functionalized with various amine-reactive groups.[31] The dendrimer was reacted with 5-fold excess FI (FITC) to generate G5-FI containing an average of 5 FI/dendrimer molecule. ^{1}H NMR and UV−visible spectral analysis showed absorbance at λ_{max} 500 nm specific for FI and confirmed the presence of 5 mol of FI/mol of dendrimer. The acetamide-capped, thiol-containing conjugate (**C6**, Figure 1) was found by UV−visible spectroscopy and by ^{1}H NMR analysis to contain an average of 2 thiols/dendrimer. The final purified product (**C9**) was analyzed by PAGE, with a 4−20% Tris−glycine gel and Tris−glycine−SDS running buffer. Antibody, antibody−dendrimer conjugate, and control dendrimer without antibody were applied on the gel along with molecular weight standards. Pure antibody appeared as a sharp band, whereas the conjugate moved relatively slower and appeared as a diffused band (probably due to partial charge neutralization of the dendrimer and the increased molecular weight of the conjugate). No band for the dendrimer was visible in the purified conjugate. UV and NMR analysis showed an average of 5 mol of FI/dendrimer molecule. The R_f value of the conjugate corresponded to a molecular weight of 200 000, indicating an average of 1 dendrimer/antibody.

Two different dendrimer−antibody conjugates, G5-FI−60B and G5-FI−PA, were used for in vitro binding studies. The conjugates bound to the corresponding antigen-expressing HL-60 and LNCaP cells in a time-dependent fashion (Figure 2). The control dendrimer G5-FI that lacked the

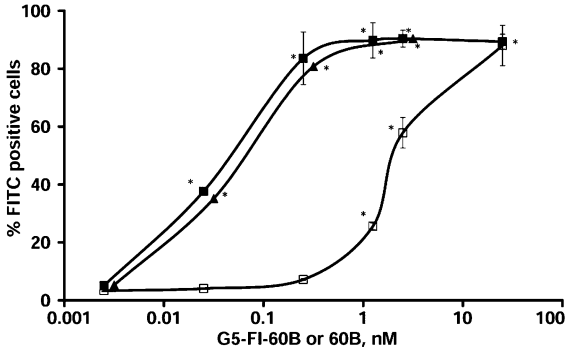

Figure 3. Dose-dependent binding of G5-FI−60B and free 60B on HL60 cells. HL60 cells were treated with different concentrations of G5-FI−60B or free 60B for 1 h at 4 °C. For treatment with the secondary antibody, cells were rinsed and treated with 62.5 nM anti-IgG-FITC for an additional 30 min, and the fluorescence of rinsed cells was measured in a flow cytometer. (□) G5-FI−60B; (■) G5-FI−60B + anti-IgG-FITC; (▲) 60B + anti-IgG-FITC. The values are mean ± SE obtained from seven independent experiments, with each experiment performed in 1−3 cell samples. Asterisks indicate $p < 0.05$ vs the control cells.

targeting antibody molecules failed to bind to the cell lines (Figure 2). The conjugate G5-FI−60B bound to the antigen-expressing cells in a dose-dependent fashion (Figure 3). The binding of the secondary antibody anti-IgG-FITC shifted the G5-FI−60B binding curve to the left to coincide with the dose−response curve obtained for free antibody detected similarly by the secondary antibody (compare □ and ■ in Figure 3). The concentration of G5-FI−60B needed to obtain similar counts of FI-positive cells was 10-fold lower in the presence of the secondary antibody than in its absence (Figure 3). This could be due to the large excess of FI on the secondary antibody as compared to that present in G5-FI−60B alone. The maximum binding fluorescence of G5-FI−PA was about 1.5−2-fold lower than that of G5-FI−60B. Confocal microscopic analysis confirmed the binding and showed evidence for cellular internalization of the dendrimer conjugates (Figure 4).

The receptor specificity of the conjugates was examined by testing the binding in control cells that do not express antigens and by competition for binding of the dendrimer with free antibody, as shown in Figure 5. The Jurkat cell line (CD14-negative) and PC3 (PSMA-negative) failed to bind the corresponding antibody conjugates. In addition, the binding of the conjugates was reversed by excess free antibody added prior to the dendrimer conjugates (Figure 5). These data show the specific receptor-mediated binding of the conjugates to the corresponding antigen-expressing cells. At 2.5 nM G5-FI−60B, free 60B inhibited the binding of the dendrimer in a dose-dependent fashion with 50% inhibition occurring at ∼6 nM 60B. This suggests that the affinity for binding of the antibody was not diminished by its conjugation to the dendrimer. Two different batches of G5-FI−60B preparation gave similar cellular binding characteristics, showing the reproducibility of the synthetic procedure (results not shown).

We have utilized a two-photon optic fiber fluorescence (TPOFF) detection system as a suitable method to quantify targeted fluorescent dendrimers in vitro and in vivo.[25,30] The conjugate G5-FI−60B gave a TPOFF fluorescence spectrum

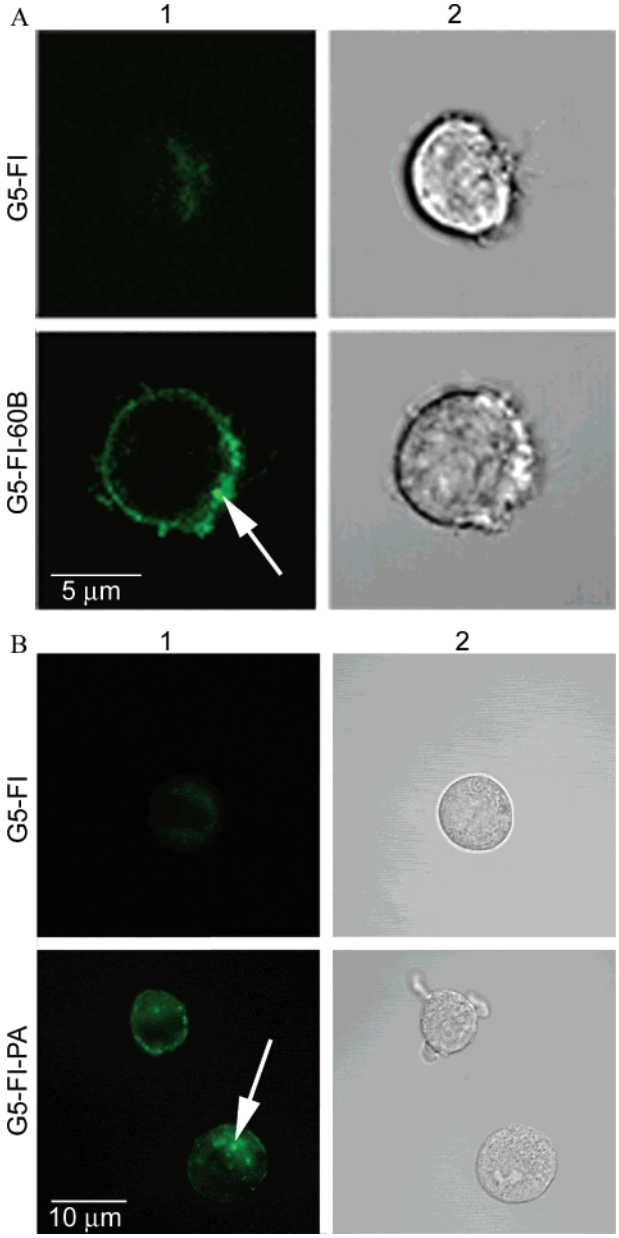

Figure 4. Confocal microscopic analysis of G5-FI−Ab-treated cells. (A) HL60 cells were incubated with 12.5 nM G5-FI or G5-FI−60B for 1 h at 4 °C and then rinsed, and confocal images were taken. Panels 1 and 2 respectively represent the FITC fluorescence and light images taken in the same cell. The arrow indicates the binding of the conjugate on the cell surface at 4 °C. (B) Confocal images of LnCaP cells treated with 50 nM G5-FI or G5-FI−PA for 1 h at room temperature. Other conditions are as given above. The arrow indicates internalization of the conjugate at room temperature. Similar results were obtained for 3−4 other cell samples.

similar to that of free FI (Figure 6B). The standard curve for the conjugate gave linear TPOFF counts at 50 to 5000 nM conjugate concentrations, with an average value of 600 counts for a 100 nM solution. The binding of G5-FI−60B to the HL-60 cells was verified by TPOFF fluorescence analysis in the cell pellets. There was a 20-fold increase in the TPOFF counts in cells treated with G5-FI−60B as compared to the control cells treated with G5-FI, and the binding was completely blocked by excess free 60B (Figure 6A). Comparable results were obtained when the cells from the same experiment were analyzed by TPOFF or by flow

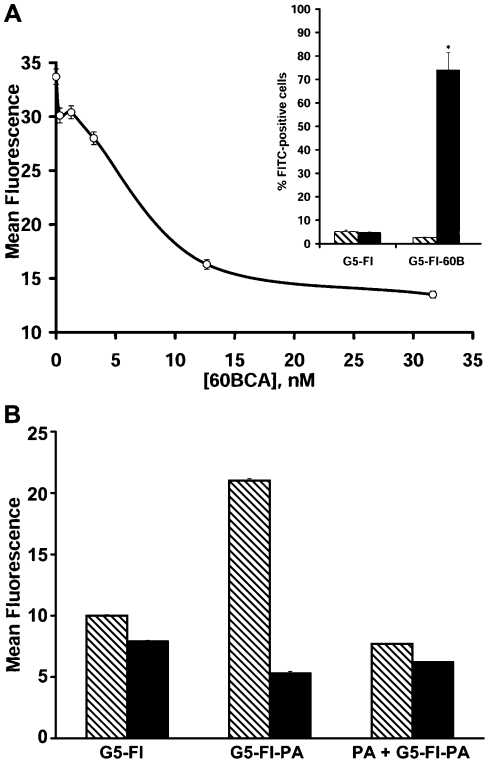

Figure 5. (A) Competition of 60B and G5-FI−60B for binding on HL60 cells. HL60 cells were incubated with 2.5 nM G5-FI−60B in the presence of different concentrations of free 60BCA antibody for 1 h at 4 °C, and the fluorescence of rinsed cells was taken in a flow cytometer. Inset: Comparison of the binding of G5-FI or G5-FI−60B (2.5 nM) in CD14-positive HL60 (solid bars) and CD14-negative Jurkat cells (hatched bars). Other conditions are as given above. (B) Reversal of the binding of G5-FI−PA (25 nM) by a 10-fold excess of free PA in PSMA-positive LnCaP cells (hatched bars) and PSMA-negative PC3 cells (solid bars). The free antibodies were added 1 min before the addition of the dendrimer conjugates. The values are mean ± SD for the cell populations from a single experiment. Similar results were obtained in a separate experiment. Inset: mean ± SE of triplicates. Asterisk indicates $p < 0.05$ vs control cells.

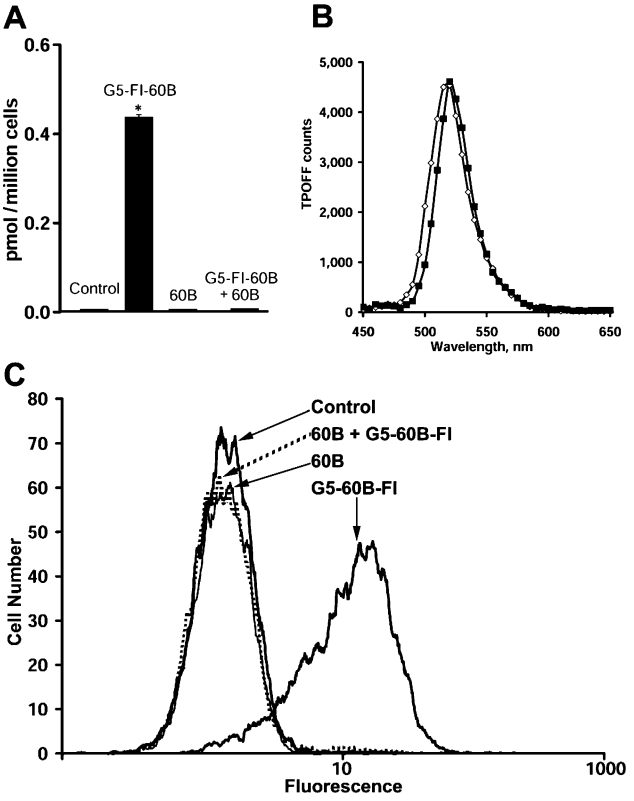

Figure 6. (A) HL60 cell association of G5-FI−60B, measured by a TPOFF probe. Cells were treated with 50 nM G5-FI−60B for 4 h at RT, in the presence or absence of a 20-fold excess of free 60B. The cells were rinsed and TPOFF counts taken in one portion of the cells by inserting the fiber into the cell pellet. The counts were converted to picomoles, by use of the TPOFF counts of standard G5-FI−60B (solid bars). The data show the mean ± SE of 25−30 readings taken in different areas of the cell pellet from a single experiment. Similar results were obtained in a separate experiment. Asterisk indicates $p < 0.05$ vs control cells and cells pretreated with 60B. (B) TPOFF spectra of free FI (○) and G5-FI−60B (■). The fiber probe was placed in 1 μM each of the dye solutions in PBS, pH 7.4, and the emitted fluorescence was scanned and counted in a photon counter. (C) Flow cytometric FL1 fluorescence taken in a second portion of the cell suspension used in panel A.

cytometry (Figure 6C). From the TPOFF counts of standard solutions of G5-FI−60B and the cell volume, the number of molecules of the conjugate associated with HL-60 cells at saturating G5-FI−60B levels was calculated to be about 265 000 molecules.

Discussion

In this report we show the successful synthesis and in vitro targeting of PAMAM dendrimer−antibody conjugates, using two different antibodies in antigen-expressing cell models. The binding of the dendrimer conjugate to cells was demonstrated by three different methods: flow cytometry, confocal microscopy, and TPOFF analysis. The confocal microscopic analysis showed evidence of the presence of the dendrimer−antibody in the cytoplasm. These data raise the possibility that the G5−Ab platform can be exploited as a delivery agent for drugs and other molecules.

The observations that the G5−Ab conjugates bound to the antigen-expressing cells in a time- and dose-dependent fashion, that the control dendrimer G5-FI failed to bind to the CD14-expressing cells, and that the G5-FI−Ab failed to associate with control cells that do not express the corresponding antigens all indicate specific receptor-mediated binding of the conjugate. The competition between the binding of the conjugates and the free antibodies further confirmed the specificity of binding. This competition study (Figure 5) also demonstrated that the antibody's affinity for binding was not affected by its conjugation to the dendrimer. The ability to conjugate dendrimers to antibodies without altering binding was further supported by the similar binding curves obtained for the free antibody and the dendrimer conjugate, as measured by the binding of a secondary antibody (Figure 3). This indicates that the antigen binding site of the antibody is not blocked by the dendrimer when an average of one dendrimer is coupled per antibody molecule. The retention of the binding affinity of an antibody following conjugation to dendrimer has been previously reported.[22]

Confocal microscopic analysis showed cellular internalization of the G5-FI−Ab conjugate, in a manner similar to other dendrimer conjugates using folic acid-targeted G5-FI−FA[24] or other dendrimer−antibody conjugates such as G5−6Tamra-PSMA[31] and G5−6Tamra-Herceptin (an un-

published observation) for targeting, showing the applicability of the dendrimer−Ab conjugate for in vivo targeting and drug delivery. Given the molecular size of the dendrimer−antibody conjugate and the lack of a receptor that takes the bound complex into the cell, internalization may appear to be a difficult process. Studies from our laboratory with three different antibody conjugates suggest some degree of internalization for each of the G5−Ab conjugates. Nonetheless, unlike the targeting through a small molecule with an internalized receptor such as folic acid, in vivo targeting by use of an intact antibody may have potential limitations due to poor tumor penetration and sequestration in the RES when administered intravenously. These problems may be partially overcome by the application of dendrimer conjugates of antibody fragments such as the single-chain Fv, which is smaller and less antigenic as compared to the intact antibody.[32]

We have developed TPOFF as a detection system to quantify fluorescence accurately in cells and in tissues.[25,30] TPOFF analysis was performed for one of the antibody conjugates, G5-FI−60B. The TPOFF emission maximum of 520 nm was retained in the G5-FI−60B conjugate, and identical concentrations of FI and the dendrimer conjugate gave similar fluorescence intensities. As there were 3−4 molecules of FI/dendrimer molecule, these results indicate the quenching of FI fluorescence in the dendrimer complex. Such fluorescence quenching has also been observed by dendrimer conjugation with other dyes such as 6-Tamra and Deep Red (results not shown). The TPOFF probe analysis showed an association of a maximum of 0.45 pmol of the dendrimer conjugates per million cells over a period of 4 h. Assuming that one antigen molecule is engaged in the G5-FI−60B binding and internalization of the antigen−antibody complex, the number of CD14 binding sites at saturating dendrimer concentrations is approximately 265 000. This value falls between the previously reported CD14 sites for the binding of the free 60bca in human monocytes (116 000) and in the vitamin D3-induced THP-1 leukemic cell line (700 000−1 000 000).[26]

In summary, this work demonstrates the targeting of two different PAMAM dendrimer−antibody conjugates in vitro. These data suggest the applicability of PAMAM dendrimers as a suitable macromolecule for the specific delivery of molecules such as a drug, targeted via an antibody. The development of a dendrimer conjugate, where an antibody or an antibody fragment serves as the targeting agent and containing multiple functions, is promising for combining cancer imaging and tumor-targeted drug delivery in cancer therapy.

Acknowledgment. This work was supported in part by the National Cancer Institute Grant N01-CM-97065-32 and by the NIH (SPORE) grant 1P50CA6568. We gratefully acknowledge technical assistance from Antonia Eliason, James Beals, and Alina Kotlyar.

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BM049704H