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A novel high throughput quantum dot-based fluorescence assay for quantitation of virus binding and attachment†

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Abstract

Quantum dots (QDots) are fluorescent semiconductor nanocrystals with a narrow emission spectrum, high quantum yield, and excellent photostability. These unique properties of QDots have been utilized to develop a fluorescent binding assay using biotinylated human T cell leukemia virus type 1 (biot-HTLV-1) conjugated with streptavidin-coated Qdots that enabled both qualitative and quantitative analyses of viral binding. The specificity and linearity of the assay was demonstrated utilizing T cells, the primary HTLV-1-susceptible cell population. Furthermore, differential binding of HTLV-1 was analyzed in additional cell types of clinical relevance including primary CD4⁺ and CD8⁺ T cells, dendritic cells (DCs), monocytes, bone marrow progenitor cells, and epithelial cells. DCs exhibited maximum binding affinity when compared to other examined cell types except the Jurkat and SUP-T1 T cell lines. Finally, blocking antibodies directed against a putative HTLV-1 receptor on DCs; DC-SIGN (dendritic cell-specific ICAM-3-grabbing non-integrin), were utilized to study the inhibition of HTLV-1 binding to target cells. Overall, these results demonstrated that this novel high throughput assay can be utilized to study the binding of a biotinylated virus and has implications for screening of viral binding inhibitors as well as host membrane proteins that may serve as receptors for viral entry.

Keywords

HTLV-1; quantum dot; viral binding assay

1.Introduction

Viral binding and attachment to a host cell membrane, while seemingly simplistic, is a complex area of research for a wide range of viruses. It is often viewed as the first step in infection, whereby a virion is able to attach to a target cell, fuse to the cell membrane, and deliver the contents of the capsid to the cytoplasm of the newly infected cell. The exact mechanism of binding to a host cell varies between viruses and is usually determined by the composition of attachment proteins located within the viral and cellular membranes. The population of cells

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infected by a virus and the establishment of infection are primarily dependent on virus binding and attachment mechanisms. By studying these mechanisms, a greater understanding of viral pathogenesis and identification of therapeutic targets can be achieved.

Current methods for detecting viral binding employ fluorophore-conjugated antibodies directed against a protein of interest for the optical detection of viral binding (Dhawan et al., 1991; Inghirami et al., 1988). Occasionally, radioactive labels are also utilized for the quantitative estimation of binding (Hubbard, 2003). However, organic fluorophores conventionally used for labeling nucleotides and proteins have poor photostability, narrow excitation bandwidth, and overlapping emission profiles in multiplexed applications. Recently developed quantum dots (QDots) are fluorescent semiconductor nanocrystals composed of a cadmium selenide (CdSe) core that can overcome the spectral drawbacks of organic fluorophores and the hazardous effects of radioactive labels (Fig. 1). Their nanoscale size (approximately 20 nm in diameter) causes the phenomenon known as the 'quantum confinement effect' which occurs in semiconductor nanocrystals due to the physical confinement of Coulomb correlated electron-hole bound pairs called excitons (Arya et al., 2005). Such nanocrystals absorb photons across a very wide wavelength range but emit only at a characteristic emission wavelength, displaying a narrow emission spectrum determined by the size and composition of the nanocrystal core. These properties make QDots excellent candidates as biological markers, particularly in the extracellular environment. QDots have an additional shell of zinc sulfide (ZnS) encasing their core that further enhances the optical properties, reduces photochemical bleaching, and increases the quantum yield (Arya et al., 2005). The core-shell material is further coated with an amphipathic polymer making the particle water miscible (Tokumasu and Dvorak, 2003). Additionally, polymerization with different substances enables QDots to expand their functionality to a broad range of applications including cell staining and biological imaging (Cognet et al., 2003; Mitchell, 2001; Roth, 1996), DNA detection (Jovin, 2003; Klarreich, 2001), cell surface receptor identification (Seydel, 2003), and immunoassays of immunoglobulin G (Koster and Klumperman, 2003; Taton, 2003). However, their use in biology is still in infancy, with no report describing the use of QDots to assess viral binding and entry. In this study, the use of QDots has been described for the first time, to develop a high throughput quantitative viral binding assay utilizing human T cell leukemia virus type 1 (HTLV-1) as a model pathogen.

HTLV-1, the first human retrovirus discovered, is the causative agent of many ailments, most notably adult T cell leukemia and HTLV-1-associated myelopathy/tropical spastic paraparesis (Gallo, 2005; Poiesz et al., 1981). Despite over 10 million people infected worldwide with HTLV-1, the mechanisms of disease pathogenesis are not yet completely understood. However, there have been numerous studies correlating host and viral factors to disease progression (Barmak et al., 2003; Grant et al., 2002). Hence, understanding of virus attachment and entry and the characterization of cell surface receptors will be vital in limiting further spread of infection. HTLV-1 infection by cell-to-cell contact is more efficient than cellfree infection (Clapham et al., 1983; de Rossi et al., 1985; Fan et al., 1992; Yamamoto et al., 1982); however, studies pertinent to virus binding need to be performed with purified virus in order to model critical events during early infection when virions first encounter host cells and begin to adhere to them. Therefore, in this study, cell-free preparations of HTLV-1 were utilized and conjugated to QDots through a well-characterized biotin-streptavidin reaction.

The process of biotinylation has been previously used to detect binding of several viruses (Dhawan et al., 1991; Inghirami et al., 1988). The most common substance used to conjugate with biotin is streptavidin. There is a high affinity constant between the glycoprotein avidin and the vitamin biotin, causing the formation of a biotin-streptavidin complex which can be used to indirectly conjugate QDots to biological molecules (Bayer and Wilchek, 1980). In this study, streptavidin coated QDots have been used to develop a simple and quantitative viral

binding assay capable of high throughput applications to detect differential binding of HTLV-1 to various pathologically relevant cell types and to conduct screening of small molecule inhibitors of viral attachment. The potential high throughput application of this assay is demonstrated by screening of a number of antibodies directed against a putative HTLV-1 receptor DC-SIGN (dendritic cell-specific ICAM-3-grabbing non-integrin).

2. Materials and Methods

2.1. Cells lines

Jurkat (clone E6-1, Cat. No. TIB-152), SUP-T1 (Cat. No. CRL-1942), U-937 (Cat. No. CRL-1593.2), TF-1 (Cat. No. CRL-2003), VK-2 cells (clone E6E7, Cat. No. CRL-2616) were obtained from American Type Culture Collection (Manassas, VA). B-THP-1, B-THP-1/DCSIGN, and P4R5 (Cat. Nos. 9940, 9941, and 3942, respectively) cell lines were obtained from NIH AIDS Research and Reference Reagent Program (Bethesda, MD). These cell lines were maintained in RPMI-1640 (ATCC) supplemented with 10% fetal bovine serum (FBS) (Hyclone, Logan, UT), penicillin (100 µg/ml), and streptomycin (100 µg/ml) (Mediatech, Herndon, VA), as previously described (Morner et al., 1999; Wu et al., 2004). TF-1 cells were cultured in the presence of recombinant human GM-CSF (2 ng/ml; eBioscience, San Diego, CA). VK-2 cells were maintained in keratinocyte-serum free medium (Invitrogen, Carlsbad, CA) with human recombinant epidermal growth factor (0.1 ng/ml, Sigma Aldrich, St. Louis, MO), bovine pituitary extract (50 µg/ml, Sigma), and additional calcium chloride (44.1 µg/ml, Sigma).

2.2. In vitro generation and culture of primary human cells

Primary human cells were generated from peripheral blood mononuclear cells (PBMCs) of healthy individuals using a standardized protocol. Briefly, CD4⁺ and CD8⁺ T cells were purified from monocyte depleted PBMCs by using EasySep negative selection enrichment cocktail (StemCell Technologies, Vancouver, Canada). Dendritic cells (DCs) were differentiated from highly purified CD14⁺ monocytes as described (Ahuja et al., 2006) and maintained in complete RPMI medium supplemented with recombinant human granulocyte macrophage colony stimulating factor (GM-CSF, 50 ng/ml, PeproTech; Rocky Hill, NJ) and interleukin-4 (IL-4, 10 ng/ml, PeproTech).

2.3. Biotinylation of HTLV-1

Sucrose density gradient purified HTLV-1 was purchased from Advanced Biotechnologies Inc. (Columbia, MD). The preparation included 4.6×10^{11} virus particles/ml in 1.2 mg/ml of total protein as determined by Pierce protein quantitation method using bovine serum albumin standards. HTLV-1 was biotinylated using a method previously described for studying adherence of HTLV-1 and other viruses (Dhawan et al., 1991). Briefly, 1 ml of cell-free HTLV-1 was incubated with 0.1 ml of dimethyl sulfoxide containing N-hydroxysuccinimido-biotin (1 mg/ml, Sigma) for 4 hr at room temperature. Free biotin was removed by dialysis against several changes of phosphate-buffered saline (pH 7.4) at 4°C. Biot-HTLV-1 was diluted in FBS-RPMI medium and stored at -80°C. A similar quantity of native virus was subjected to the biotinylation process but biotin was omitted. This preparation served as a mock control in the binding assay.

2.4. Quantum dot-based binding assay

A schematic representation of the quantum dot-based binding assay is shown in Fig. 1. Cells (1×10^6) were first resuspended in 200 µl assay buffer (Hank's-buffered saline solution supplemented with 3% FBS and 0.02% sodium azide) and incubated for 45 min with biot-HTLV-1 at 4 and 37°C in 96-well U-bottom plates (BD Labware, Franklin Lakes, NJ).

Unbound virus particles were removed by washing cells three times with assay buffer followed by centrifugation (Beckman Coulter 1500R) at 1500 rpm at 4°C for 10 min and subsequent aspiration of supernatant via multi-channel pipette to avoid loss of cell pellets. Cells were then incubated with strep-QDot 605 (10 nM, Quantum Dot Corporation, Hayward, CA) for an additional 30 min at 4°C. Subsequent to washing as described above, cells were fixed using 1% paraformaldehyde for 20 min. The strep-QDot:biot-HTLV-1 complex was detected by fluorescence at an excitation/emission wavelength of 400/605 nm using the Micromax microwell plate reader on the FluoroMax-3 spectrofluorometer (Jobin Yvon Inc., Edison, NJ) and data was obtained from Datamax software version 2.2 (Jobin Yvon Inc.). Background fluorescence was subtracted from each sample and mean fluorescence was obtained by taking the average of samples performed in duplicate.

2.5. Confocal microscopy

For confocal microscopy, samples from the binding assay were plated onto Biocoat poly-D-lysine-coated 8-well tissue culture slides (BD Labware) at 5×10^4 cells per well. Cells were allowed to adhere for 2-4 hr at 37°C and sequentially fixed with 2% paraformaldehyde (10 min) and 4% paraformaldehyde (20 min) at room temperature. Nuclei were stained with DAPI using VECTASHIELD mounting media containing DAPI (Vector Laboratories, Burlingame, CA). Images were obtained with a dual laser confocal system (Leica TCS SP2) connected to a microscope (Leica DRME) using the Leica LCS software package.

2.6. Competitive inhibition of binding

To perform competition analyses of HTLV-1 binding, target cells were incubated with biot-HTLV-1 (0.125 µg) along with increasing concentrations (0.031-0.5 µg) of non-biotinylated HTLV-1 for 45 min at 4°C. The unbound virus was removed by washing and cells were subsequently combined with strep-QDot. Binding was quantitated as described above.

2.7. Screening of blocking antibodies against a putative HTLV-1 receptor DC-SIGN

To study the inhibition of HTLV-1 binding to DCs, target cells (B-THP-1, B-THP-1/DC-SIGN, or DCs) were pre-incubated for 20 min with DC-SIGN-reactive antibodies: Clone 120507 (20 µg/ml, R&D Systems, Minneapolis, MN), Clone 120516, Clone 120526, Clone 120531, Clone 120604 (20 µg/ml, NIH AIDS Reagent program), DCN46 (20 µg/ml, BD Biosciences, San Jose, CA), SC-20 (20 µg/ml, SantaCruz Biosciences, Santa Cruz, CA), and AZN-D1 (20 µg/ml, kindly provided by Dr. T. B. Geijtenbeek, Department of Molecular Cell Biology, Vrije Universiteit Medical Center, Amsterdam, Amsterdam, The Netherlands).

3. Results

3.1. Dose-dependent binding of HTLV-1 to T cells

To examine the linearity of the binding assay, Jurkat cells (CD4⁺ T cells, representative of the primary infected cell population in vivo) were subjected to binding with increasing concentrations of biot-HTLV-1 (0.031-0.5 µg) at both 4 and 37°C (Fig. 2A). Cells incubated with biotin and QDots alone served as the negative controls for the experiment. At 4°C and lower concentrations of virus (< 0.1 µg), there was a rapid, linear, dose-dependent increase in the observed mean fluorescence (A400/605). A plateau in fluorescence intensity was observed with 0.125 µg of virus (45,086 fluorescence units [F.U.]). At 37°C, the observed mean fluorescence increased in a linear manner, but at a much slower rate compared to 4°C, with an average decrease of 72%. The fluorescence intensity reached a plateau at 0.25 µg of virus (36,722 F.U.), a concentration 2-fold higher than that required at 4°C. These results indicated that at 4°C, 0.125 µg of biot-HTLV-1 represented the optimal concentration and was used in further experimentation as it was within the linear region of the binding reaction and

represented a concentration that did not saturate binding sites completely, allowing for accurate assessment of the kinetics of viral binding. Samples from the binding assay were also visualized by confocal microscopy after staining with DAPI (blue) for nucleus detection. The conjugation of biot-HTLV-1 with strep-QDot enabled the direct imaging of virus (red) on the cell surface (Fig. 2B) indicating that QDots can be used for both quantitative and qualitative assessment of binding.

3.2. Competitive inhibition of viral binding

Competitive inhibition studies were performed to determine the specificity of the assay. Jurkat cells with biot-HTLV-1 (0.125 μ g) were reacted with increasing concentrations (0.031-0.25 μ g) of non-biotinylated (native) HTLV-1. A progressive inhibition in biot-HTLV-1 binding was observed in the presence of native HTLV-1 (Fig. 3). Specifically, there was a 70% decrease (P -value of 0.0119) in fluorescence intensity when the quantity of non-biotinylated virus was 2-fold less than biot-HTLV-1 (24,631 F.U.) and an 84% decrease (13,439 F.U., P -value of 0.0074) when biot-HTLV-1 and native HTLV-1 were present in equal quantities. At native HTLV-1 quantities greater than biot-HTLV-1, the observed fluorescence intensity decreased further, indicating very low levels of biot-HTLV-1 binding. These results demonstrated that the observed binding was specific to HTLV-1.

3.3. HTLV-1 binding affinity to clinically relevant cell-types

Once the assay was standardized and examined for linearity and specificity, the binding affinity of HTLV-1 to clinically relevant cell types was examined for the presence of receptors or attachment molecules specific to HTLV-1. The differential binding of biot-HTLV-1 to T cell lines (Jurkat and SUP-T1), primary T cells (CD4⁺ and CD8⁺), DCs, monocytic cell lines (U-937 and B-THP-1), a bone marrow-derived progenitor cell line (TF-1), and epithelial cell lines (VK-2 and P4R5) were examined as indicated in Fig. 4. T cells represent the primary susceptible population *in vivo* while progenitor cells, monocytes, and dendritic cells have also been reported to be susceptible to infection by HTLV-1 (Ali et al., 1993; Makino et al., 1999) and were hence assessed for their relative binding affinity to HTLV-1. HTLV-1 has been shown to play a role in the pathogenesis of cutaneous T cell lymphoma (Zucker-Franklin, 2001), therefore, vaginal keratinocyte (VK-2) cells were also examined with respect to binding. The P4R5 cell line, a derivative of the epithelial cell line HeLa engineered to express HIV receptor (CD4) and co-receptors (CCR5 and CXCR4) were examined to determine if receptors for HIV-1 also played a role in HTLV-1 binding. Jurkat cells demonstrated maximal fluorescence intensity when compared to all the cell lines (82,633 F.U.). The monocytic cell lines U-937 and B-THP-1 (14,808 and 11,016, F.U. respectively) and TF-1 progenitor cells (8,535 F.U.) exhibited lower fluorescence intensities, indicating reduced capability of these cells to initiate cell-free virus infection under the experimental conditions. Both VK-2 and P4R5 cells exhibited very low levels of binding, indicating low affinity of HTLV-1 to epithelial cells. Furthermore, P4R5 cells demonstrated that receptor and co-receptor requirements for HIV-1 and HTLV-1 are different in accordance with a previously published report (Kannagi et al., 1991). Among the primary cells, DCs demonstrated the highest fluorescence intensity (48,977 F.U.) while CD4⁺ and CD8⁺ T cells exhibited relatively lower fluorescence intensities (20,342 and 16,201, F.U. respectively).

3.4. Blocking antibodies directed against a putative HTLV-1 receptor on DCs

To demonstrate the high throughput utility of the binding assay for the screening of novel virus binding inhibitors, an array of antibodies directed against a putative receptor for HTLV-1 on DCs named DC-SIGN (dendritic cell specific ICAM3-grabbing non-integrin) was screened. These antibodies have been previously characterized with respect to the binding and transmission of HIV-1 by DC-SIGN (Wu et al., 2002). The screening of antibodies was

performed on primary DCs as well as B cells expressing very low endogenous levels of DC-SIGN (B-THP-1) or transduced to express high levels of DC-SIGN (B-THP-1/DC-SIGN) (Wu et al., 2004). The fluorescence intensities in the absence or presence of DC-SIGN blocking antibodies when pre-incubated with B-THP-1 or B-THP-1/DC-SIGN cells are shown in Fig. 5A. B-THP-1/DC-SIGN cells exhibited a 5-fold increase in fluorescence intensity as compared to B-THP-1 cells, which was statistically significant (P -value of 0.0031). However, in the presence of blocking antibodies, B-THP-1/DC-SIGN cells exhibited a significant reduction in fluorescence intensity. The most effective antibodies with respect to blocking virus binding were Clone 120507, Clone 120516, Clone 120531, DCN46, and SC-20, exhibiting 52%, 57%, 45%, 49% and 49% reductions in fluorescence intensities, respectively. All of these antibodies are DC-SIGN-specific. Clone 120604, an L-SIGN (homolog of DC-SIGN)-specific antibody, did not effectively block binding, demonstrating fluorescence intensities on a par with fluorescence intensities in the absence of antibodies. Clone 120526, which has been shown to be crossreactive with DC-SIGN and L-SIGN (Wu et al., 2002), also did not block virus binding effectively. Levels of fluorescence intensity observed in the presence of AZN-D1, another DC-SIGN-specific antibody that was previously characterized to inhibit binding of HIV-1 envelope glycoprotein gp120 to DC-SIGN (Geijtenbeek et al., 2000), exhibited no significant effect on HTLV-1-binding to B-THP-1 cells.

The fluorescence intensities in the absence and presence of DC-SIGN blocking antibodies when pre-incubated with DCs are shown in Fig. 5B. The mean fluorescence intensities from DCs in the absence and presence of blocking antibodies (55,038 and 23,274, F.U. respectively) exhibited a 58% reduction in binding. All DC-SIGN-specific antibodies Clone 120507, Clone 120516, Clone 531, DCN46, and SC-20 exhibited statistically significant inhibition of virus binding. L-SIGN-specific antibody, Clone 120604 exhibited a 20.3% decrease in fluorescence intensity, but this reduction was deemed insignificant. Clone 120526, which is cross-reactive to DC-SIGN and L-SIGN (Wu et al., 2002), effectively blocked virus binding with an observed reduction of 58%. Fluorescence intensity in the presence of AZN-D1 was reduced by 34%, indicating greater effectiveness of AZN-D1 in blocking the binding of HTLV-1 to DCs as compared to DC-SIGN-transduced B cells.

4. Discussion

While QDots are, in principle, able to share many of the same applications as organic fluorophores such as membrane labeling, fluorescent microscopy, antibody tagging, and flow cytometry, their strength lies in the fact that they can perform these tasks with greater optical output in a narrower emission spectrum when compared to standard fluorophores. Since QDots are biologically non-reactive in their native conformation, they must be conjugated to some form of intermediate biochemical such as streptavidin to utilize them in most biological assay systems. The uncoated semiconductor nanocrystal alone cannot be attached to biochemicals or proteins under normal conditions. QDot 605 was selected to label the virus since it fluoresces in the range of 580-640 nm, providing spectral separation from DAPI commonly used to label cell nuclei. QDot 605 also presents a tradeoff between spectral intensity and size, with size and intensity both increasing with wavelength. Although QDot 605 is slightly larger in diameter compared to QDots emitting at lower wavelengths, its higher fluorescence intensity lends to greater sensitivity in estimation of binding of virus particles.

The quantum dot-based binding assay utilized in this report employs streptavidin-coated QDots to label biotinylated HTLV-1 (biot-HTLV-1) particles bound to the surface of target cells. Unbound biot-HTLV-1 and strep-QDot controls showed negligible fluorescence in all cases, ruling out interference from the non-specific binding of biot-HTLV-1. The autofluorescence from unbound cells was also accounted for and subtracted from each sample. Assays to assess the linearity of the reaction demonstrated that the labeling of virus particles does not affect

virus binding to the target cells as evidenced from the dose-dependent increase in fluorescence intensity at lower quantities of native virus. The plateau in fluorescence intensity observed at 4°C could be due to saturation of binding sites due to the monovalent or multivalent reversible binding of receptors by virus particles. Although the rate of increase in fluorescence intensity at 37°C was lower, a higher end-point saturation level in fluorescent intensity was achieved. The reduced rate of change in fluorescent intensity may be attributed to greater diffusion of receptor molecules from the more energetic conditions at 37°C, thereby increasing the number of multivalent reversible bonds between a virus particle and several receptor molecules; in effect reducing the number of available free receptor molecules. The lower rate of increase in fluorescence intensity with increase in temperature could also be due to increased endocytosis of QDots at 37°C. QDots have been shown to be easily endocytosed when incubated with cells (Jaiswal et al., 2003), hence the quantity of QDots available for binding virions is reduced, thereby having an effect on viral binding. Because endocytosis is reduced at 4°C, greater fluorescence intensities were observed and hence 4°C was used as the temperature for further experimentation.

Competitive inhibition assays using increasing quantities of native HTLV-1 indicated that biotinylated virus exhibited a lower affinity for cellular attachment than native virus. Fluorescence intensities decreased significantly when quantities of native HTLV-1 were higher than biot-HTLV-1, clearly indicating specificity of binding between virus particles and adhesion molecules or receptors on the cell surface.

Once the linearity and specificity of the assay was confirmed, the efficiency of the assay to differentiate HTLV-1 binding to various clinically relevant cell types based on their relative binding affinity to the virus was examined. Apart from the Jurkat and SUP-T1 CD4⁺ T cell lines, maximum fluorescence intensity was exhibited by DCs as compared to other cell types including primary T cells signifying that these cells possess greater affinity for HTLV-1 and may contain one or more HTLV-1-specific receptors or attachment molecules. These results also implied that HTLV-1 may preferentially bind to DCs at the mucosal entry site, which may provide a cellular shuttle to the lymphatic system, where infection can be transferred to the primary T cell population as demonstrated in case of HIV-1 (van Kooyk and Geijtenbeek, 2003). The P4R5 cells, engineered to over-express the HIV-1 receptor CD4 and co-receptors CXCR4 and CCR5, indicated a lack of binding to HTLV-1. This could imply that although HTLV-1 and HIV-1 share some biological similarities, including their preference for infecting CD4⁺ T cells, receptor/co-receptors for HIV-1 do not have an effect on HTLV-1 binding and entry.

The receptor for HTLV-1 has remained elusive for many years until recently, when a glucose transporter GLUT-1 was demonstrated to act as an HTLV-1 receptor on T cells (Manel et al., 2003). However, despite its ubiquitous nature, GLUT-1 has not been shown to facilitate viral binding and entry of HTLV-1 into DCs. Surface molecules on immature DCs have been reported to organize into microdomains of about 200 nm in the lipid raft regions of the plasma membrane (Cambi et al., 2004). The aggregation of surface molecules and putative receptors lends credence to the possibility that HTLV-1 binding may involve the formation of multiple bonds between the virus and the host cell target. DCs express an array of receptors for pathogens, most of which fall into two categories, Toll-like receptors and C-type lectins (van Kooyk and Geijtenbeek, 2003). One of the most well characterized C-type lectins with reference to viral binding is DC-SIGN, which has already been demonstrated as a receptor for number of viruses, including HIV-1 (Geijtenbeek et al., 2000). Our recent results have indicated that DCSIGN facilitates binding, entry, and productive HTLV-1 infection of DCs (Jain and Wigdahl, unpublished observations). It has also been demonstrated that antibodies directed against DCSIGN prevent HTLV-1 fusion and infection through an ICAM (intercellular adhesion molecule on T cells)-dependent mechanism (Ceccaldi et al., 2006). Therefore, a

number of monoclonal antibodies, known to block interaction of DC-SIGN to ICAM-3 thereby preventing HIV-1 transmission, were screened with respect to inhibition of HTLV-1 binding. In accordance with the published report of DC-SIGN-mediated HIV-1 binding (Wu et al., 2002), it was observed that DC-SIGN-specific antibodies such as Clone 120507, Clone 120516, and Clone 120531 were also effective with respect to HTLV-1 binding. Two additional DC-SIGN-specific antibodies DCN46 and SC-20 exerted significant decrease in binding on both DCs and transduced B cells; however, AZN-D1 was only effective on DCs but not on B cells. While the cross-reactive antibody Clone 120526 was the most effective blocking agent relevant to HIV-1 binding, it only moderately blocked HTLV-1 binding. Furthermore, the L-SIGN specific antibody Clone 120604 did not inhibit HTLV-1 binding indicating no cross-reactivity between DC-SIGN and L-SIGN with respect to HTLV-1 binding. Additional studies with mutated DC-SIGN could facilitate the determination of epitopes that are important for HTLV-1 binding. A previous study demonstrated the involvement of the carbohydrate recognition domain of DC-SIGN in HIV-1 envelope binding (Baribaud et al., 2002). Therefore, screening of antibodies against specific DC-SIGN epitopes using a high throughput assay such as this could be useful in deciphering the mechanism of virus envelope interaction with cell surface molecules.

With the advent of nanotechnology and its subsequent application to biology, we now are able to provide more quantitative explanations of activities at the cellular and molecular level in a high throughput manner. The fluorescence-based assay that we have developed is the first report of the use of quantum dots to label virus particles for rapid and accurate quantitation of binding events occurring on the cell surface. Presently, the assay is designed for 96-well plates; however, with emerging expertise in nanofabrication techniques, it could be adapted for high throughput screening of arrays of antibodies or peptides in nano-well plates requiring picomolar quantities of reagents. The results reported herein demonstrate that our assay is quantitative, high throughput, and can be utilized to analyze the binding of any virus that can be biotinylated as well as for the screening of viral binding inhibitors, epitope mapping, and receptor studies.

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The abbreviations used are

HTLV-1	human T cell leukemia virus type 1
Biot-HTLV-1	biotinylated-HTLV-1
QDot	quantum dot
Strep-QDot	straptavidin-coated QDot
DCs	dendritic cells
DC-SIGN	dendritic cell-specific ICAM-3-grabbing non-integrin
F.U	fluorescence units

Fig 1. Schematic representation of the quantum dot-based binding assay to quantitate HTLV-1 binding to target cells. Cell-free virus preparations were biotinylated to facilitate detection with streptavidin-coated quantum dots. Virus binding to the target cells was measured by quantitative estimation of fluorescence at appropriate excitation and emission spectra.

Fig 2. Binding and imaging of HTLV-1 on Jurkat T cells using quantum dot-based binding assay. (A) Jurkat T cells (1×10^6) were incubated with increasing amounts of biot-HTLV-1 (0.031 μ g – 0.5 μ g) for 45 min at 4 and 37°C. Unbound virus was removed by extensive washing and binding of target cells was quantitated as described in Materials and Methods. Biotin and QDot alone served as negative controls and fluorescence readings were used for background subtraction. Biot-HTLV-1 exhibited a dose-dependent increase in binding to T cells and reached saturation at higher virus concentrations demonstrating the linearity of the binding reaction. Data represents mean fluorescence $\pm$ standard deviation ($\pm$ SD) from two independent experiments each performed in duplicate. (B) Samples from the binding assay were plated onto poly-D-lysine-coated 8-well tissue culture slides to directly image the strep-QDot:biot-HTLV-1 complex on the cell surface. Nuclei are stained with DAPI (blue) and biot-HTLV-1 are labeled with QDot 605 (red). Confocal microscopy demonstrated that QDot labeled biot-HTLV-1 surrounded the cell surface uniformly as indicated by white arrows, thus confirming binding.

Fig 3.

Competitive inhibition of HTLV-1 binding. Jurkat cells (1×10^6) were incubated with biot-HTLV-1 (0.125 μg) alone or in the presence of increasing concentrations (0.031-0.25 μg) of non-biotinylated virus for 45 min at 4°C. Unbound virus was removed by extensive washing and binding of target cells was quantitated as described in Materials and Methods. QDot and biotin alone served as negative controls demonstrating minimal fluorescence. A gradual inhibition in biot-HTLV-1 binding with increasing concentrations of native HTLV-1 was observed, indicating the specificity of the binding assay. Data represents mean fluorescence intensity $\pm$ SD from two independent experiments each performed in duplicate. Asterisk (*) or ** denotes a statistically significant change in fluorescence compared to basal values with $p < 0.05$ and $p < 0.01$, respectively.

Fig 4. Binding of HTLV-1 to selected pathologically relevant cell types. T cell lines (Jurkat, SUP-T1), primary T cells (CD4⁺, CD8⁺), dendritic cells (DCs), monocytic cell lines (U-937, B-THP-1), a bone marrow-derived progenitor cell line (TF-1), and epithelial cells (VK-2, P4R5) were incubated with biot-HTLV-1 (0.125 μ g) for 45 min at 4°C. Unbound virus was removed by extensive washing and binding of target cells was quantitated as described in Materials and Methods. Biotin and QDot alone served as negative controls and their fluorescence readings were used for background subtraction. Differential binding of HTLV-1 to selected cell types was observed. Data represents mean fluorescence intensity $\pm$ SD from two independent experiments each performed in duplicate.

Fig 5.

Epitope screening of a putative HTLV-1 receptor, DC-SIGN, using various blocking antibodies. Target cells: B-THP1 and B-THP-1/DC-SIGN (A) or DCs (B) were pre-incubated for 20 min with blocking antibodies (20 $\mu\text{g}/\text{ml}$) against DC-SIGN (Clone 120507, Clone 120516, Clone 120531, Clone 120526, Clone 120604, DCN46, SC-20, AZN-D1). Cells were then incubated with biot-HTLV-1 (0.125 μg) for 45 min at 4°C. Unbound virus was removed by extensive washing and binding of target cells was quantitated as described in Materials and Methods. Biotin and QDot alone served as negative controls and their fluorescence readings were used for background subtraction. Data represents mean fluorescence intensity $\pm$ SD from three independent experiments, each performed in duplicate. Asterisk (*) denotes a statistically significant change in fluorescence compared to basal values with $p < 0.01$.