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A fully human antibody to gp41 selectively eliminates HIV-infected cells that transmigrated across a model human blood brain barrier

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Abstract

Objective—Many HIV patients on cART exhibit HIV-associated neurocognitive disorders because the brain becomes a viral reservoir. There is a need for therapeutics that can enter the CNS and eradicate the virus.

Design—Radiolabeled human mAb 2556 to HIV gp41 selectively kills HIV-infected cells in vivo and in vitro. Here we tested the ability of 213Bi-2556 to cross a tissue culture model of the human BBB and kill HIV-infected PBMCs and monocytes on the CNS side of the barrier.

Methods—2556 mAb isoelectric point (pI) was determined with IEF. The ability of radiolabeled 2556 to penetrate through the barrier was studied by adding it to the upper chamber of the barriers and its penetration into the CNS side was followed for 5 hrs. To assess the ability of ^{213}Bi-2556 to kill the HIV-infected cells on the CNS side of barrier, the HIV-infected and uninfected PBMCs and monocytes were allowed to transmigrate across the barriers overnight followed by application of ^{213}Bi-2556 or control mAb ^{213}Bi-1418 to the top of the barrier. Killing of cells was measured by TUNEL and Trypan blue assays. The barriers were examined by confocal microscopy for overt damage.

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Supplementary Fig. 1 Biodistribution of ^{111}In-CHXA”-2556 and control ^{111}In-CHXA”-1418 mAbs in CD1 mice 5 and 24 hrs post systemic administration: a) uptake of ^{111}In-CHXA”-2556 and control ^{111}In- CHXA”-1418 mAbs into the brains of CD1 mice 5 and 24 hrs post systemic administration; b) uptake in the organs at 5 hrs time point; c) uptake in the organs at 24 hrs time point.

Results—The pI of ^{213}Bi-2556 was 9.6 enabling its penetration through the barrier by transcytosis. ^{213}Bi-2556 killed significantly more transmigrated HIV-infected cells in comparison to ^{213}Bi-1418 and uninfected cells. No overt damage to barriers was observed.

Conclusions—We demonstrated that ^{213}Bi-2556 mAb crossed an in vitro human BBB and specifically killed transmigrated HIV-infected PBMCs and monocytes without overt damage to the barrier.

Keywords

neuroAIDS; HAND; in vitro human blood brain barrier model; HIV-infected human PBMCs; monocytes; radioimmunotherapy; 213-Bismuth

INTRODUCTION

With the advent of combined anti-retroviral therapy (cART), people with HIV are living much longer and infection can be treated as a chronic disease. However, there are reservoirs within the body where the virus persists and continues to cause damage. Infection of the central nervous system (CNS) poses a particular challenge due to strict regulation by the blood brain barrier (BBB) which limits many cART regimens from reaching effective levels in the brain [1]. In fact, recent data indicated that even with cART regimens that penetrate the CNS, there is no decrease in HIV associated neurocognitive disorders (HAND), and these regimens might make it worse as a result of neurotoxicity [2]. HIV enters the CNS in infected monocytes shortly after peripheral exposure [3] and subsequently infects macrophages, microglia, and, to a lesser extent, astrocytes [4,5]. The infection causes release of several inflammatory mediators that damage neurons and the cells composing the BBB, specifically, astrocytes [6] and brain microvascular endothelial cells (BMVECs) [7]. These mediators also recruit additional monocytes into the CNS. Since the introduction of cART in 1996, many cART-treated people have shown near complete suppression of HIV replication. The incidence of moderate to severe dementia fell from about 7% in 1989 to only 1% in 2000 [8]. Despite this remarkable improvement in severe neurologic impairment, the prevalence of HAND continues at very high rates [9,10] with as many as 53% of all HIV infected people having mild to moderate neurocognitive impairment [11]. HAND greatly contributes to HIV morbidity, as does the presence of the “silent” reservoirs in CNS. Thus, this “hidden epidemic” [12] creates an urgent need for a treatment capable of eliminating HIV from the CNS.

Radioimmunotherapy (RIT) was developed as cancer treatment and is currently approved for clinical use in patients with non-Hodgkins lymphoma [13,14]. Our laboratory has pioneered the use of RIT for infectious diseases [15] and demonstrated the ability of RIT to kill HIV-infected human peripheral blood mononuclear cells (PBMCs) [16]. Recently we identified a fully human monoclonal antibody (mAb) 2556, that is directed towards a highly conserved epitope on the gp41 surface protein found both on virus particles and infected cells [17]. In vitro tests demonstrated that 2556 bound to the immunodominant domain (cluster 1) of gp41 shared across all subtypes of HIV within clades A to H, and was able to outcompete the native mAbs to gp41 in HIV+ serum for binding to infected cells. When SCID mice carrying HIV-infected PBMCs were treated with 2556 mAb radiolabeled with

alpha particles-emitting radionuclide 213-Bismuth (^{213}Bi), significant killing of HIV-infected cells was achieved without hematologic toxicity to the animals [17]. ^{213}Bi-2556 mAb also selectively killed infected cells from the blood of HIV-infected individuals on various cART regimens as well as from cART-naïve ones [18]. Here we describe studies designed to examine the ability of 2556 mAb to penetrate an *in vitro* human BBB model and to kill specifically HIV-infected cells with the goal of eliminating HIV from the CNS.

METHODS

Antibodies and radiolabeling

Human IgG1 mAb 2556 to gp41 was generated from B cells of an HIV-1 infected individual using hybridoma technology [17]. Production of 2556 was scaled up in CHO cells transfected with immunoglobulin genes isolated from 2556 hybridoma cells by Goodwin Biotechnology (Plantation, FL). The control isotype matched human mAb 1418 that binds to parvovirus B19 [19] was made at the New York University School of Medicine, and a human anti-CTLA4 mAb ipilimumab was purchased from Bristol-Myers Squibb, New York, NY. MAb 2556 was conjugated to a bifunctional ligand N-[2-amino-3-(p-isothio-cyanatophenyl)propy1]-trans-cyclohexane-1,2-diamine-N,N′,N″,N‴,N″-pentaacetic acid (CHXA") (Macrocyclics, San Antonio, TX) that enables subsequent radiolabeling with trivalent metals. Two to fifty initial molar ratio of CHXA" to mAb was used for conjugation as in [20] and its immunoreactivity towards gp41 was assessed by gp41 ELISA as in [17]. The number of CHXA" molecules attached to the mAb as a result of the conjugation was determined by Yttrium-Arsenazo III assay as in [21]. The control mAbs 1418 and ipilimumab were conjugated with the molar excess of CHXA" selected as the optimal one for 2556 mAb as above. 111-Indium (^{111}In) was purchased from Perkin Elmer. ^{213}Bi was eluted from a ^{225}Ac/^{213}Bi generator provided by the Institute for Transuranium Elements, Karlsruhe, Germany [22]. MAbs conjugated with CHXA" were radiolabeled with ^{213}Bi and ^{111}In in [23].

Isolation of human PBMCs and CD14+ monocytes and culturing of CD14+CD16+ monocytes

Anticoagulated blood was obtained from healthy donor leukopacks from the New York Blood Center. PBMCs were isolated by Ficoll-Paque centrifugation. A portion of the PBMC were cultured at 2×10^6 cells/mL in polypropylene tubes and activated with IL-2 at 10 U/mL and PHA at 5 μg/mL for 48 hours to obtain activated T cells and monocytes. The remaining PBMCs were used to isolate CD14+ cells using the EasySep Human CD14+ isolation kit (StemCell Technologies, Vancouver, BC). The freshly isolated CD14+ monocytes were cultured non-adherently in Teflon flasks at 2×10^6 cells/mL for 3 days with 10 ng/mL M-CSF (Peprotech, Rocky Hill, NJ) in supplemented RPMI to facilitate monocyte maturation and to yield monocytes that were highly enriched for CD14+CD16+ cells [24]. We showed previously that this mature subset of monocytes is the population that can be HIV infected and preferentially crosses the BBB [24–26].

Infection of PBMCs and CD14+CD16+ enriched monocytes

Cell-free viral inoculum was obtained from the NIH AIDS Research and Reference Reagent Program (Germantown, MD). HIV_{ADA}, an R5 strain that primarily infects human monocytes/macrophages, was used. Twenty ng/mL of virus was added to the suspensions of 2×10^6 cells and incubated for 2 hours for PBMCs and overnight for isolated monocytes. The cells were then cultured for an additional 6 days (PBMCs) or 48 hours (monocytes). The supernatant was collected and the levels of p24 were measured by p24 ELISA (Advanced Bioscience Laboratories, Kensington, MD). The infectivity of mature monocytes varies extensively due to the fact that these are all primary cells derived from independent donors. Our p24 values of culture supernatant range from 200 pg-10 ng/ml.

Human in vitro BBB model and permeability testing

The protocol for use of human tissues has been approved by the Albert Einstein College of Medicine Committee on Clinical Investigation. The in vitro model of the human BBB was cultured as previously described [27] and a schematic illustrating the model is shown in Fig 1. To assess permeability of the BBB, the co-cultures were rinsed with phenol-red-free DMEM. Four hundred μl 10%FBS/phenol-red-free DMEM were placed in the bottom of a well and the insert was added. Two hundred μl 0.45% BSA conjugated to Evans blue dye were added to the top of the insert and incubated for 30 minutes at 37°C, 5% CO_2. After incubation, the inserts were removed, media from the lower chamber was collected and read spectrophotometrically at 620 nm for presence of BSA-Evans blue dye as an indicator of permeability.

The isoelectric point (pI) determination

The pI of naïve 2556 and CHXA”-conjugated 2556 mAbs as well as of control human mAbs 1418 and ipilimumab was determined by isoelectric focusing (IEF). The pI was determined under non-denaturing conditions without dithiothreitol (DTT) reducing agent. The sample buffer contained 0.5M Urea, 4% CHAPS, 0.5% IPG buffer.

MAbs penetration through the human BBB model

To determine whether the 2556 mAb could cross the BBB, the following series of experiments were performed using CHXA”-conjugated 2556 and 1418 mAbs radiolabeled with ^{111}In. The radiolabeled mAbs were added in 200 μl aliquots to the endothelial side of co-cultures (Fig. 1). Five μl samples were taken from the astrocyte side of the barriers at hourly intervals for 5 hours. Four barriers per group were used. The amount of radioactivity that passed through the barrier was counted in a gamma counter and percent of the initial activity of the added sample that passed through the barrier was calculated.

Comparative biodistribution of ^{111}In-labeled 2556 and 1418 mAbs in mouse brain

All animal experiments were approved by the Albert Einstein College of Medicine Institute for Animal Studies. Groups of eight 6–8 weeks old CD1 female mice were injected intraperitoneally with either 50 μCi ^{111}In-labeled 2556 or 1418 control mAbs. At 5 and 24 hours post-injection, 4 mice from each group were sacrificed, their whole brains and major

organs carefully removed, weighed, and radioactivity was counted in a gamma counter. The percentage of injected dose per gram tissue (ID/g) was calculated.

Killing of PBMCs and monocytes that transmigrated across the BBB with RIT

PBMCs were isolated and infected as described above. Media on the astrocyte side contained 100 ng/ml CXCL12 to mediate chemotaxis of lymphocytes and monocytes [26]. Three hundred thousand of either HIV-infected or non-infected PBMCs were added to the endothelial side of the co-cultures and incubated overnight. Any remaining cells were aspirated off and 200 μl media containing either 30 μCi ^{213}Bi-CHXA"-2556, unlabeled ("cold") 2556, 30 μCi ^{213}Bi-CHXA"-1418 control mAb, or no antibody, were added to the endothelial side of the co-cultures. The number of cells that had migrated to the astrocyte side and remained viable was counted at 24, 48 and 72 hours following treatment with RIT. The "no treatment" group was used as a control to account for baseline cell death. The number of apoptotic cells at each time point was assessed with TUNEL assay (Trevigen). Six barriers per condition were used, and the experiment was performed 2 independent times.

Monocytes were isolated and infected as described above. Media on the astrocyte side of the co-cultures contained 200 ng/ml CCL2 to mediate maximal chemotaxis [26, 28]. Three hundred thousand HIV-infected or non-infected monocytes were added to the endothelial side of the co-cultures and incubated overnight. Any remaining cells were aspirated off and 200 μl media containing varying doses of ^{213}Bi-CHXA"-2556 (0, 10, 30 and 50 μCi) were added to the endothelial side and an additional 200 μl media were added to the astrocyte side at this time. The monocytes that transmigrated were allowed to remain at the bottom of the 24-well cell culture dish for a total of 72 hours after treatment with RIT. At this time trypsin or TrypLE Express (Invitrogen) was used to lift the cells that had adhered to the dishes. Cells were spun and re-suspended in equal volumes (100 μl) for cell counting with Trypan blue. The number of live cells was calculated. In the subsequent series of experiments the transmigrated infected or non-infected monocytes were treated with 30 μCi ^{213}Bi-CHXA"-2556 or ^{213}Bi-CHXA"-1418 control mAb to assess the specificity of RIT towards gp41 expression on infected cells. Four or five barriers per condition were used. The experiment was performed 3 independent times.

Confocal microscopy of RIT-treated barriers

HIV infected or uninfected monocytes were allowed to transmigrate through the barriers overnight as described followed by addition to the top of the barrier of either 10 or 30 μCi ^{213}Bi-CHXA"-2556, or 30 μCi ^{213}Bi-CHXA"-1418 or NH_4OAc buffer for 4 hours. The co-cultures were washed, fixed with PFA, mounted in OCT medium and cut on a cryostat into 10 μm section. Sections were incubated with fluorescent-labeled phalloidin to stain for actin, and with DAPI (diamidinophenylindole) – for nuclei. The experiment was performed 2 independent times.

Statistics

Comparisons between treatment groups were made using a one-tailed Student's t-test and statistical significance was considered when P value was <0.05.

RESULTS

Bi-labeled mAb to gp41 has a high isoelectric point

We determined the optimal number of CHXA" ligand molecules to be attached to 2556 mAb to enable radiolabeling with ^{213}Bi. While the increase in the number of ligand molecules per mAb molecule (ligand to protein ratio) favors radiolabeling, too many ligand molecules could decrease immunoreactivity. Fig. 2a shows the dependence of the 2556 immunoreactivity towards gp41 on the number of the conjugated CHXA" molecules. Ten molar excess of CHXA" over 2556 mAb in the conjugation reaction resulted in the ligand to protein ratio of 3.8, and 50% immunoreactivity when compared to that of unmodified 2556. Based on these results, all subsequent ligand conjugations for 2556 and control mAbs used 10 molar excess of CHXA" over mAb.

Next we determined by IEF the pIs of 2556 and 1418 isotype-matched control mAb in their naïve form, conjugated to CHXA" ligand, and labeled with Bi. Although the penetration of large molecules through the BBB is limited, highly basic proteins that are characterized by high pI values are able to enter the CNS through absorptive-mediated transcytosis [29]. This process is not saturable because no specific transport receptor is required. It has been demonstrated that increasing pI of a protein increases its transcytosis-mediated entry into CNS several folds [reviewed in 29]. Interestingly, fully human mAbs have inherently higher pIs than murine mAbs [30], e.g. human mAb to gp41 was reported to have a high pI of 8.8 [31]. The pIs were found to be 8.6–9.2 for naïve mAb 2556, 9.2–9.6 for CHXA"-conjugated 2556, and 9.6–9.9 for Bi-CHXA"-2556 (Fig. 2b). For comparison, bovine serum albumin (BSA), that cannot penetrate the human BBB, has a pI of 4.8. The pI of the control 1418 mAb also increased after conjugation with CHXA", and subsequent attachment of Bi resulted in the final pI reaching 9.15 (Fig. 2c). To test whether the increase in the pI as a result of labeling with Bi^{+3} is a general trend for human mAbs, we measured the pI of ipilimumab, a human anti-CTLA4 mAb currently approved by FDA for melanoma treatment, and observed the same effects of conjugation and Bi labeling on the pI (Fig. 2d). Thus, we hypothesized that both Bi-labeled 2556 and control 1418 mAbs would penetrate the human BBB.

2556 and control human mAb penetrated through an in vitro model of the human BBB

To address whether the antibodies penetrated the BBB, we used ^{111}In-CHXA"-2556 and compared its penetration through the human BBB to that of 1418 control mAb. ^{111}In emits non-cytotoxic gamma rays and is commonly used as a radiotracer in quantitative and imaging studies in place of therapeutic radionuclides such as ^{213}Bi. It carries the same +3 ionic charge that makes the overall charge of the mAbs labeled with Bi and In identical. The penetration was time dependent with 6% of the total amount of ^{111}In-CHXA"-2556 mAb penetrating the barrier by 5 hrs (Fig. 2e). We did not evaluate further time points, as by 5 hrs all ^{213}Bi activity would decay. ^{111}In-CHXA"-1418 mAb also penetrated through the barrier, although somewhat less (4% penetration by 5 hrs) which can be explained by its slightly lower pI. However, the difference in penetration was not statistically significant ($P>0.05$). We also conducted a biodistribution experiment in mice to determine whether the CHXA"-2556 could cross the BBB in vivo. The uptake of ^{111}In-CHXA"-2556 in the brains

of CD1 mice was slightly higher than that of ^{111}In-CHXA”-1418 mAb at both 5 and 24 hrs post-administration without reaching statistical significance (Supplemental Fig. S1a). Both 2556 and 1418 quickly cleared from the major organs due to low cross-reactivity between mammalian tissues and mAbs to viral proteins (Supplemental Fig. 1b,c). We did not analyze any possible inflammatory responses in the mice, although both the gp41-specific and control antibodies are fully human antibodies and can potentially cause some mouse anti-human antibody response (MAHA). However, in human patients there should not be any immune response to a fully human antibody. The vast majority of the antibodies currently approved for clinical use have been made in chimeric, humanized or fully human forms specifically for the purpose of avoiding immune response.

HIV-infected PBMCs and monocytes were selectively killed by ^{213}Bi-CHXA”-2556 post transmigration through the model BBB

We assessed the efficacy and the specificity of ^{213}Bi-CHXA”-2556 mAb in killing infected PBMCs that transmigrated across the BBB. HIV-infected and uninfected PBMCs were added to the top of the BBB and allowed to transmigrate through the BBB overnight. ^{213}Bi-CHXA”-2556 or control ^{213}Bi-CHXA”-1418 were then applied to the endothelial side of the barriers. At 48 hrs post addition of the antibodies, approximately 1/3 of infected PMBCs treated with 30 μCi ^{213}Bi-CHXA”-2556 were undergoing apoptosis as per TUNEL assay, In contrast, the numbers of apoptotic cells in all control groups were at background levels (Fig. 3a,b) ($p=0.008$). To study the killing of mature monocytes by the radiolabeled mAb, the cells were added to the top of the barriers, allowed to transmigrate overnight, and increasing doses of ^{213}Bi-CHXA”-2556 were added to the top of the BBB. In cultures that received 10 μCi ^{213}Bi-CHXA”-2556, 96% of cells were alive in the uninfected cultures compared to 44% cells alive in the HIV-infected group ($p<0.005$) (Fig. 3c). At 30 μCi, 87% of the uninfected cells were alive versus 62% of infected cells ($p<0.009$). In the 50 μCi group we observed a greater amount of non-specific cell apoptosis due to the high concentration of radiation in the samples. Despite this, the killing of infected cells was significantly more pronounced than that of the non-infected cells ($p=0.01$). These results are encouraging as monocytes are more radioresistant than lymphocytes, which represent most of the PBMC population [32]. In addition, in vivo experiments with HIV-infected PBMCs in SCID mice [16,17] demonstrated neither hematopoietic nor general toxicity from RIT, thus confirming RIT specificity. We also compared killing of infected and uninfected monocytes by ^{213}Bi-CHXA”-2556 and control ^{213}Bi-CHXA”-1418. Infected or uninfected monocytes transmigrated across the BBB overnight as described above and were exposed to 30 μCi ^{213}Bi-CHXA”-2556 or ^{213}Bi-CHXA”-1418 control. ^{213}Bi-CHXA”-2556 killed infected monocytes but not uninfected ones ($p=0.006$) while control ^{213}Bi-CHXA”-1418 did not kill either infected or uninfected monocytes (Fig. 3d).

Lastly, we evaluated the integrity of the barriers post RIT. We tested the permeability of the co-cultures after experimental treatments with radiolabeled 2556 and 1418 mAbs using Evans blue dye coupled to BSA as described in the Methods section. All co-cultures were impermeable to the dye that was demonstrated spectrophotometrically by background level absorption at 620 nm. This indicated that treatment with the mAbs did not promote BBB permeability. To confirm these observations, we performed the confocal microscopy of the

endothelial side of the barrier which showed that RIT did not cause overt damage to the barriers (Fig. 4) thus providing data on the safety of RIT towards the BBB. This is an important observation, as the preservation of BBB integrity after the penetration of the radiolabeled mAb is crucial for the safety of RIT in the clinic.

DISCUSSION

The use of targeted therapy with α-particles emitters in oncology is burgeoning worldwide. This is driven by the advantages of α-emitters over β-emitters, including very specific targeting of the diseased cells due to the α-particles' short 50–80 μm tissue range, and increased killing efficiency due to high linear energy transfer. This results in a controlled therapeutic modality with minimal normal tissue effects [33]. RIT with α-emitters does not depend on the oxygenation status of the tumor or its resistance to chemotherapy and external beam radiation therapy, as recently demonstrated by ^{213}Bi-labeled peptide-induced remission in patients with neuroendocrine tumors refractory to β-radiation [34]. In particular, ^{213}Bi-labeled mAbs have been used in multiple clinical trials for several oncologic indications, and have demonstrated efficacy without major side effects [35,36]. There is also clinical experience in using ^{213}Bi for brain tumors: ^{213}Bi-labeled substance P was used in the setting of inoperable gliomas, causing no cognitive impairment in treated patients [37]. Our pre-clinical findings in treating mice with cryptococcal infection in the brain with ^{213}Bi-labeled mAbs demonstrated that killing of microbial cells was not accompanied by neurological or systemic side effects [38].

Encouraged by the clinical successes of ^{213}Bi-labeled antibodies in oncology and by our RIT results in the experimental models of infectious diseases including HIV, we performed this study designed to examine the ability of 2556 mAb to penetrate through an *in vitro* human BBB model and to kill specifically HIV-infected cells, with the goal of eliminating HIV from the CNS. This is critical to any cure as CNS remains a long-lived protected reservoir for the virus. Our results provide the proof of efficacy and selectivity of killing HIV-infected PBMCs and monocytes which transmigrated across the BBB by radiolabeled 2556 mAb. The short 46 min half-life of ^{213}Bi leads to its complete decay within 4 hrs and makes it appealing with respect of minimizing side effects on the BBB. Future studies will also examine other alpha-emitting radionuclides with longer physical half-lives which can deliver radiation to the infected cells for several days. Potential candidates include astatine-211, actinium-225, radium-223 or thorium-227. In this regard, radium-223 chloride (physical half life 11.4 days) is FDA approved for treatment of certain types of prostate cancer. Overall, current investigational cures for HIV, such as gene disruption and bone marrow transplantation [39, 40], do not address the major impact of neuroAIDS. Our results indicate that RIT with radiolabeled mAbs directed towards HIV gp41 shows promise as a strategy to eliminate HIV-infected cells, both systemically and specifically in the CNS, and warrants further investigation. We have recently demonstrated [18] that the ART-treated HIV infected PBMCs still express, albeit decreased, levels of gp41 on their surface, that enables the specific killing of such ART-pretreated cells with RIT. In fact, the combination of ART and RIT was more effective in eliminating such cells than either modality alone. In regard to the truly latent cells, it might be possible to use RIT of HIV in CNS in conjunction with latency-reversing agents.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

AMF, LL, MV, DW, RB, ED, EE, AG performed the experiments; SZP, MG, AM, FB provided the reagents; AMF, ED, JWB analyzed the results and wrote the manuscript.

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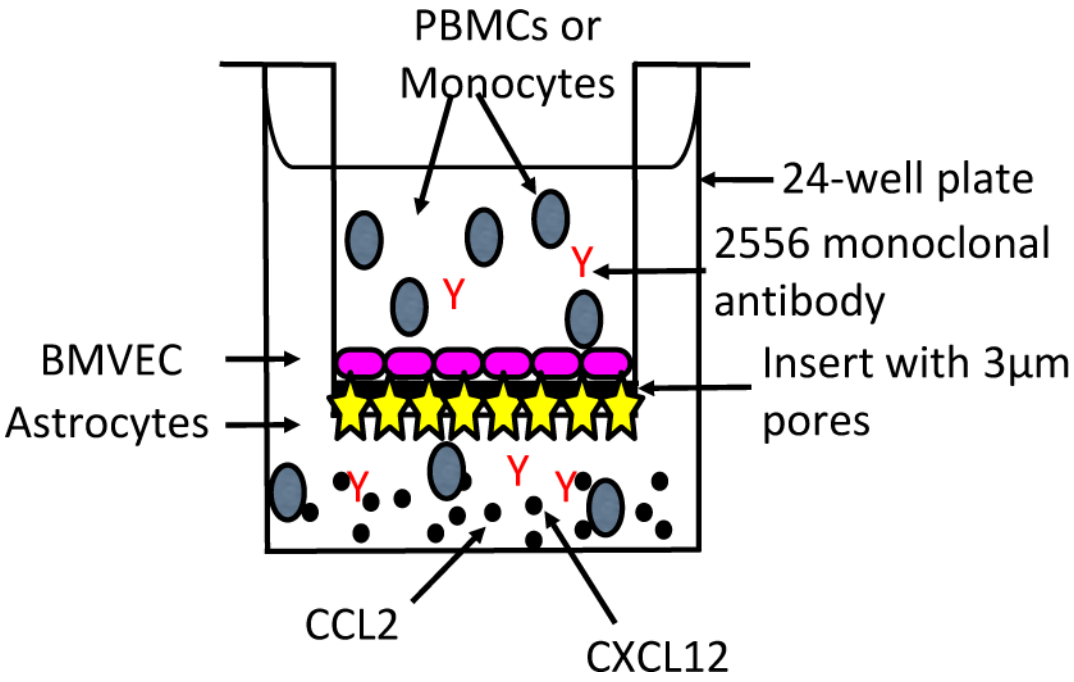

Fig. 1.
Schematic of the *in vitro* human BBB used in this study.

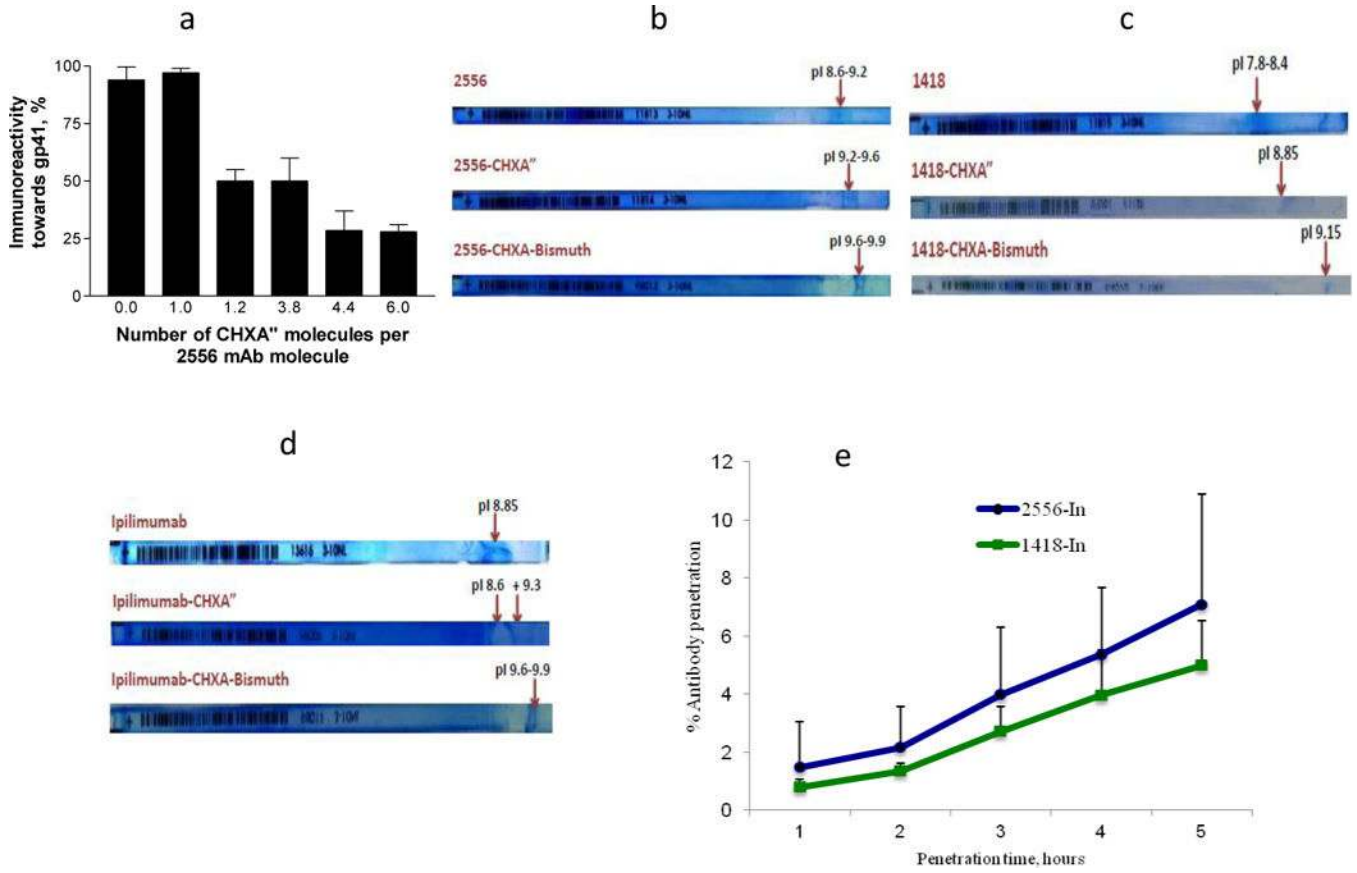

Fig. 2.

Physical and biological characterization of CHXA”-conjugated 2556 mAb. a) dependence of the 2556 immunoreactivity on the number of CHXA” ligand molecules conjugated to a 2556 molecule. The immunoreactivity of the conjugates towards HIV gp41 was determined by gp41 ELISA. The number of CHXA” molecules per 2556 molecule was determined by Yttrium-Arsenazo III assay; b) IEF of naïve 2556, CHXA”- 2556 and Bi-CHXA”-2556 mAb; c) IEF of naïve 1418, CHXA”-1418 and Bi-CHXA”-1418 mAb; d) IEF of naïve ipilimumab, CHXA”- ipilimumab and Bi-CHXA”- ipilimumab. Non-denaturating IEF conditions were utilized; d) penetration of ^{111}In-CHXA”-2556 and control ^{111}In-CHXA”-1418 mAbs through the human BBB model.

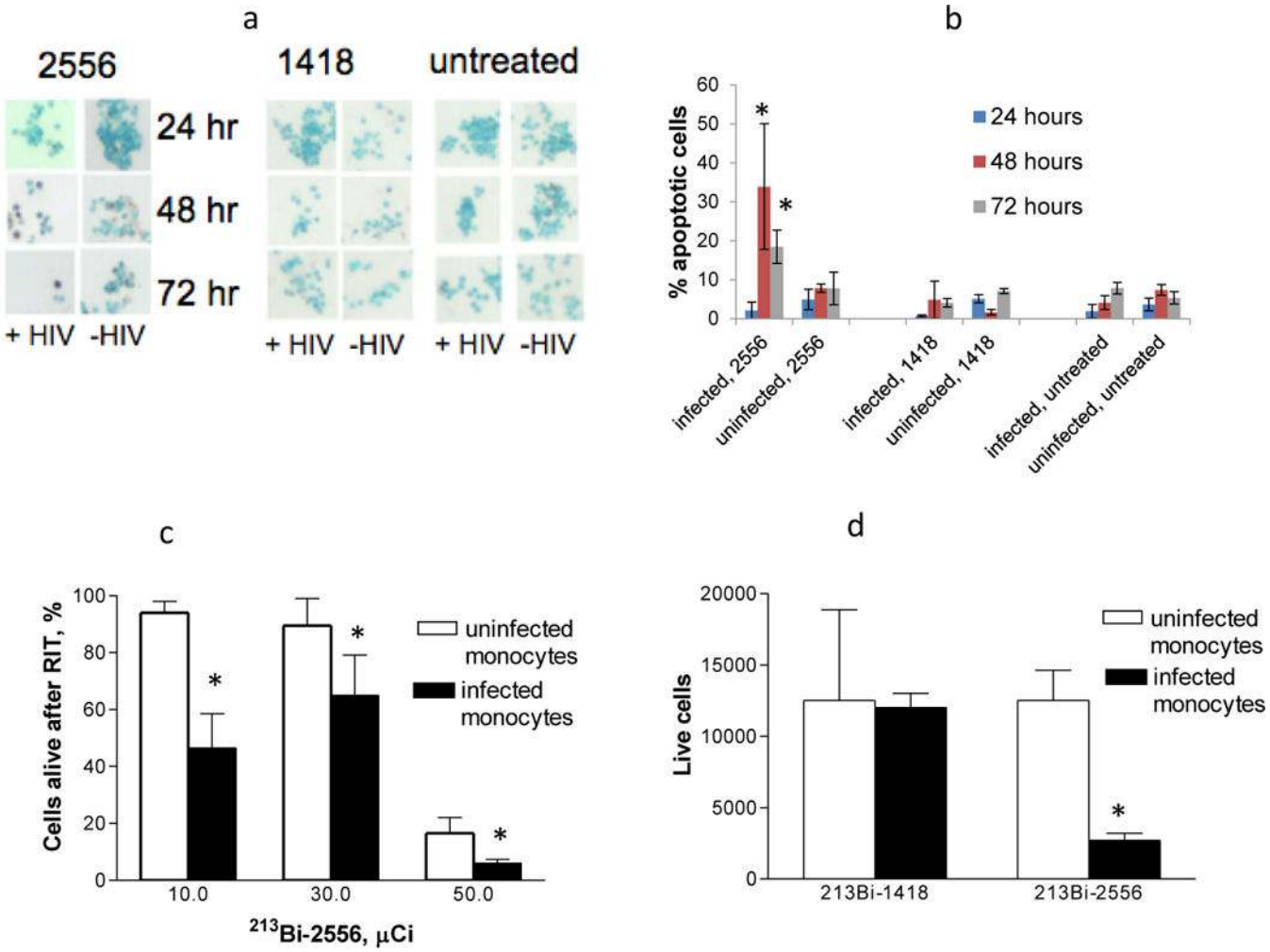

Fig. 3.

Killing of HIV-infected PBMCs and monocytes with ^{213}Bi-CHXA”-2556 mAb that transmigrated across an vitro human BBB model: a) TUNEL staining at 24, 48 and 72 hrs of HIV-infected and uninfected PBMCs that transmigrated across the in vitro BBB in response to CXCL12 and treated with 30 µCi ^{213}Bi-CHXA”-2556 mAb, or 30 µCi ^{213}Bi-CHXA”-1418 mAb, or untreated. Apoptotic cells stained brown; b) percentage of apoptotic cells in transmigrated HIV-infected and uninfected PBMCs treated as above. Experiment was performed twice, the * denotes statistical significance; c) dose dependent killing of HIV-infected and uninfected monocytes which transmigrated across the in vitro BBB in response to CCL2 and were treated with ^{213}Bi-CHXA”-2556 mAb. The percentage of live cells was determined by dividing the cell numbers in the treated groups by the cell numbers in the untreated group; d) specific killing of infected monocytes with 30 µCi ^{213}Bi-CHXA”-2556 and absence of killing of infected and uninfected monocytes with 30 µCi ^{213}Bi-CHXA”-1418 control mAb. Experiments were performed 3 times, the * denotes statistical significance.

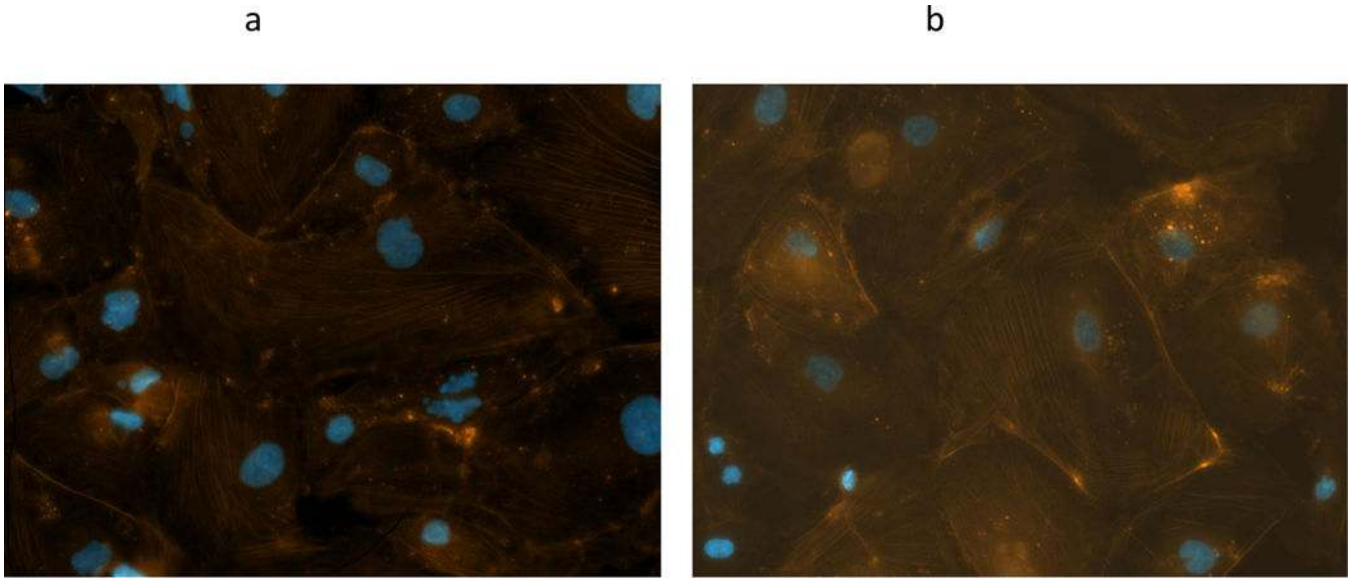

Fig. 4.

Confocal microscopy of the in vitro human BBB showing the absence of overt damage to the BBB from RIT: a) untreated barrier; b) barrier treated with 30 μCi ^{213}Bi-CHXA”-2556. Actin was stained with fluorescent-labeled phalloidin, nuclei (blue) were stained with DAPI. Experiment was performed twice.