Neutralizing and Targeting Properties of a New Set of α4β7-Specific Antibodies Are Influenced by Their Isotype

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Abstract: The homing of lymphocytes to the mucosa is mainly controlled by α4β7 integrin, and it is amplified during gut chronic inflammation, as occurs with HIV and/or inflammatory bowel diseases. We designed and applied an improved immunization strategy based on an innovative selection process to isolate new α4β7--lymphocyte–specific monoclonal antibodies that are able to prevent their migration into inflamed gut tissues and/or to counteract HIV infection in vitro. First, 5 monoclonal antibodies (1 IgA, 1 IgM, and 4 IgGs) were selected based on their capacity to recognize α4 or β7 homodimers and α4β7 heterodimers in transfected human cells. Their ability to block gp120/α4β7 or MAdCAM-1/α4β7 interactions was then measured in vitro with human T and B lymphocytes. In vitro, the anti-α4β7 IgA isotype was found to have the highest affinity for the α4β7 heterodimer, and it significantly reduced HIV replication in retinoic acid–treated α4β7–/CD4– human T cells. This α4β7–specific IgA also displayed a high avidity for human and mouse α4β7 lymphocytes in both mouse and human inflammatory colitis tissues. These new antibodies, and in particular those with mucosa-targeting isotypes such as IgA, could therefore be potential novel therapeutic tools for treating HIV and inflammatory bowel disease.

Key Words: HIV, GALT, integrin α4β7, HIV-neutralizing mAb, gut, gp120, MAdCAM-1

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INTRODUCTION

The homing of lymphocytes to mucosal tissues, such as gut-associated lymphoid tissue (GALT) and the endocervix (which are the primary locations of activated memory lymphocytes) is mainly controlled by integrin α4β7.1,2 With mucosal chronic diseases, such as inflammatory bowel disease (IBD) and HIV infection, inflammation contributes to damage and destruction of the epithelium. Depletion of GALT lymphocytes by administration of anti-α4β7 monoclonal antibodies (mAbs) has been reported to improve clinical scores for patients with Crohn’s disease (CD)3–5 and ulcerative colitis.6 Moreover, intravenous administration of natalizumab or vedoluzimab has been reported to limit proinflammatory responses by increasing the Th17/Treg ratio and by blocking lymphocytes homing to inflammatory areas. Subcutaneous administration of etrolizumab, which is an mAb that targets the β7 subunit of α4β7 and ααβ7, is currently undergoing phase III trials.5 During the first step of HIV infection, the virus targets the integrin α4β7.6 At day 10 postinfection, α4β7–/CD4– T cells contain higher levels of SIV RNA than other CD4+ T cells.7 A high frequency of α4β7+ CD4+ T cells in the rectal mucosa greatly increased susceptibility to SIV infection.8 The SIV or HIV gp120 envelope protein (Env) has been reported to specifically bind to α4β7 on T and B cells.6,9 Gp120 binding to α4β7 could be blocked in vitro and in vivo by treatment with anti-α4β7 mAb (eg, Act-1) or anti-αα mAb (eg, 2B4 and natalizumab). Several anti-α4β7 and anti-αα mAbs have been reported to reduce and/or delay infection of human retinoic acid (RA)-treated α4β7–/CD4– T cells in vitro by SF162 primary isolate.6,10,11 The Env of other HIV strains with the ability to bind to α4β7 or which contain an LDV tripeptide sequence were not inhibited by anti-α4β7 mAbs.12,13 Recently, intravenous administration of anti-α4β7 mAb in macaques before SIV challenge was found to result in significant protection of the animals and a reduction of viral load and proviral DNA integration in the gut.14 Five years after infection, the animals remained healthy, with CD4+ T cell counts of up to 500 cells per milliliter, whereas all the control animals died within 2 years.11,13 The combination of antiretroviral therapy and anti-α4β7 mAbs in SIV-infected monkeys leads to effective control of viremia and the restoration of CD4+ cells, persisting even after termination of the therapy.16 The use of biological agents targeting integrins is becoming more widespread with IBD, and new therapeutic indications may arise, such as HIV infection. In light of this, we decided to generate new and more potent anti-α4β7 integrin mAbs that

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are amenable to alternative administration routes suitable to both IBD and HIV infection. Here, we compare various mAb isotypes raised against the native transmembrane conformation of human α4β7 integrin with the ability to target inflammatory lymphocytes in intestinal tissues and to block gp120/α4β7 interactions. An innovative immunization protocol was used to generate these potent new mAbs. We identified 5 particularly relevant candidates that are specific for α4 or β7 homodimers and/or α4β7 heterodimers that vary in their ability to block binding of gp120 to α4β7 or to delay in vitro HIV replication in human RA-treated α4β7+ CD4+ T cells.

MATERIALS AND METHODS

α4β7 Human Cells

RPMI 866 (European Collection of Authenticated Cell Cultures) is a human B cell line expressing high levels of α4β7 integrin (in the order of 60,000 receptors/cell). Human Embryonic Kidney (HEK) 293 cells (American Type Culture Collection) were used for transient transfections with Lyvvec (InvivoGen, Toulouse, France). Plasmids p-α4 (GeneCard#ITGA4) and/or p-β7 (GeneCard#ITGB7) were provided by GeneCopoeia (Rockville) and used to transiently transfect HEK 293 cells. As described previously, Human distal duodenum (Labonor, France), and cut into serial sections of 7 µm, were obtained from biopsies of individual patients who had undergone upper duodenal endoscopy. Biopsies were also obtained from informed and consenting patients with CD or healthy individuals who had undergone upper duodenal endoscopy.

Production of Anti-α4β7 mAbs

Balb/C mice were immunized with various α4β7 immunogens. On d0 and d14, a combination of 0.5 × 106 RPMI 866 cells and 8 µg of recombinant human α4β7 integrin (R&D systems, Minneapolis, MN) emulsified with complete Freund adjuvant (Sigma) was administered intraperitoneally. On d21, the mice were administered 0.75 × 106 HEK cells transiently transfected with α4β7+, and they were boosted a final time on d28 with 4 µg of intravenous rHu α4β7 integrin and 0.75 × 106 α4β7+ HEK cells transiently transfected with α4β7+. Splenocytes were then harvested and fused with Sp2/0 myeloma cells, as described elsewhere. The mAbs were produced using CELLine flask (INTEGRA Biosciences, Cergy-Pontoise, France).

To purify the mAbs, ammonium sulfate solution was added to the hybridoma supernatant at 4°C for 30 minutes. After centrifugation, the pelleted material was diluted into 10 mL of 20 mM Tris and then dialyzed (ThermoFisher). The samples were then centrifuged, passed through a 0.22-µm pore size filter, and subjected to ion-exchange chromatography (QMA Hyper D; Pall Life Sciences, EastHills, NY). This chromatographic separation process was followed by BioLogic DuoFlow (Bio-Rad) chromatography. Identification of the class, subclass, and light chain types of the mouse immunoglobulins were performed with an isoelectric mouse mAb isotyping kit (Roche).

Production, Purification, and Labeling of gp120

CHO-SEC was used to produce clade B Env glycoprotein (obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH, courtesy of Dr. Carol Weiss and Dr. Judith White). To enhance the yield of secreted gp120, a clonal dilution of the CHO-SEC was performed to select the clone producing the most gp120 (20 µg/mL). Culture supernatants were harvested and purified with a galanthus nivalis column (Vector Laboratories), as described previously. To minimize avidity binding, galanthus nivalis lectin was diluted with Sepharose 4B (at a ratio of 1:4). The column was washed with 20 mM Tris (pH 8.4) buffer containing 100 mM NaCl. Elution was performed with an acidic buffer (20 mM glycine (pH 2.5), 100 mM NaCl, and 500 mM α-methylmannopyranoside), as described previously. Tris–HCl neutralization buffer (1 M at pH 8) was added to each eluted fraction. Positive fractions were pooled, concentrated (regenerated cellulose; 50,000 NMWL; Amicon; Millipore) and dialyzed against Hank’s buffered salt solution buffer. The gp120 proteins were then biotinylated (EZ-Link NHS-Biotin, Pierce) for 30 minutes at room temperature (RT) and dialyzed against Hank’s buffered salt solution to remove excess biotin. All the gp120 protein samples were then aliquoted and stored at −80°C.

Immunolabeling of GALT Biopsies

GALT tissues from mouse colitis models were used. Briefly, C57bl6 mice were provided with drinking water that contained 5% dextran sodium sulfate. Human distal duodenum biopsies were also obtained from informed and consenting patients with CD or healthy individuals who had undergone upper duodenal endoscopy.

Biopsy samples were utilized in order to block gp120/α4β7 interactions. An innovative immunization protocol was used to generate these potent new mAbs. We identified 5 particularly relevant candidates that are specific for α4 or β7 homodimers and/or α4β7 heterodimers that vary in their ability to block binding of gp120 to α4β7 or to delay in vitro HIV replication in human RA-treated α4β7+ CD4+ T cells.

α4β7-Binding Immunoassay

α4β7 and αEβ7 integrins (R&D systems) were coated at 1 µg/mL in 96 well plates (Nunc MaxiSorp, VWR). After blocking with 4% Bovine serum albumin buffer for 1 hour at 37°C, the mAbs were added for 2 hours at 37°C. Rabbit
polyclonal horseradish peroxidase antimouse Immunoglobulin against IgG, IgA, and IgM (Dako) (at 1:500) was added for 1 hour at 37°C. The optical density was measured at 450 nm (Sunrise, Teican).

Flow Cytometry-Based α4β7-Binding Assay

CD3-Vioblue, CD4 (VIT4)-VioGreen and CD8-APC-Vio770 were obtained from Miltenyi Biotec. CD45RA-PC7 and FIB504 were purchased from Beckman Coulter and from Santa-Cruz biotechnologies, respectively. Anti-α4 mAb (clone 2b4) was obtained from R&D, and Act-1 was provided by the NIH AIDS Reagent program (Division of AIDS, NIAID, NIH, courtesy of Dr. A.A. Ansari14). Staining of human CD4+ T cells was carried out in a buffer containing 10 mM HEPES/150 mM NaCl/100 mM CaCl2/1 mM MnCl2 and 5% Bovine serum albumin. To block any nonspecific binding, cells were preincubated with both mouse and human IgG for 10 minutes on ice (5 μg each per 106 cells). CD4-gp120 interaction was masked by preincubating the cells for 15 minutes with 5 μg Leu3A (BD Biosciences), as described previously.9 α4β7/gp120, α4β7/MADCAM-1, and α4β7/mAb (DDX1430, DDX1431, DDX1432, DDX1434, and DDX135) interactions were masked by preincubating the cells for 15 minutes with 5 μg of unlabeled 2B4 mAb (R&D Systems) or with 5 μg of unlabeled anti-α4β7 antibodies. Staining was performed by incubating for 25 minutes on ice with 1 μg of ligand, either biotinylated-gp120, PE-conjugated MadCAM-1 (R&D Systems), or PE-conjugated anti-α4β7 antibodies. Cells were then washed twice and incubated for 30 minutes with PE-conjugated neutravin (Pierce) and/or CD45RO fluorescence isothiocyanate mAb (BD Bioscience). Cells were washed 3 times in staining buffer and then fixed with a solution of 1% paraformaldehyde. Data were acquired with FACSCanto (BD) and analyzed using FlowJo software (Ashland). Mean fluorescence intensities were determined for the CD4+ populations.

Surface Plasmon Resonance Spectroscopy

The kinetics of antibody construct binding to α4β7 was assessed by surface plasmon resonance (SPR) spectroscopy using a Biacore 3000 biosensor (BIACORE Life Sciences, Uppsala, Sweden). Control integrins, α4β1, and α6β4, were tested in parallel. The integrins were immobilized on separate CM4 biosensor surfaces (Biacore, Inc.) using amine-coupling chemistry at a surface density of ~750 resonance units each. Increasing concentrations (ranging from 3.125 to 50 nM) of the indicated antibody constructs were passed sequentially over the sensor surfaces for 2 minutes at 25 μL/min in HEPESbuffered saline (pH 7.4) supplemented with 1 mM CaCl2 and 0.005% Tween-20, followed by a 2-minute dissociation phase. The surfaces were regenerated after each cycle by 2 brief injections of 4.5 M MgCl2 at a flow rate of 100 μL/minute. The data were evaluated using BiaEvaluation 4.1 software.

Neutralization Assays

As described previously, peripheral blood mononuclear cells were isolated from human blood, and CD4+ T cells were purified by negative selection. Purified CD4+ T cells were stimulated with 1.5 μg/mL of anti-CD3 mAb (OKT3, eBiosciences), 20 units/mL of hHu IL-2 (R&D Systems), and 10 nM of RA (Sigma). After 24 hours and then every 2 days, the medium was replaced with fresh medium containing only hHu IL-2 and RA. Upregulation of α4β7 integrin was controlled by flow cytometry. Before infection, the cells were incubated for 1 hour at 37°C with anti-α4β7 mAb at 0.0625 μg/mL. This concentration had been determined previously by pretitration assays. The cells were then incubated with virus for 12 hours at 37°C and washed twice to remove unbound HIV-1 strains.

RPMI 8866 cells were also used to deliver virus particles to CD4+ T cell, as described previously.24 Virus and RPMI 8866 cells were mixed for 1 hour at 37°C, and after 5 washes, α4β7+ CD4+ T cells were added at 37°C at a ratio of 1:1. We evaluated whether RPMI 8866 cells coated with viral particles by binding to α4β7 integrin were able to transfer them to α4β7+ CD4+ T cells. We first confirmed that the RPMI 8866 cells were not infected at d12. For all neutralization experiments, culture supernatants were collected at 3, 6, and 9 days postinfection to measure p24 antigen by the enzyme-linked immunosorbent immunoassay (InSight Diagnostics). SF162 (clade B, R5), 92US660 (clade B, R5), and 92UG029 (clade A, X4) were obtained through the NIH AIDS Reagent Program (Division of AIDS, NIAID, NIH: HIV-92US660 from the Multicenter Cohort Study, HIV-1 92UG029 from the UNAIDS Network for HIV Isolation and Characterization. HIV-1 SF162 virus was courtesy of Dr. Jay Levy25).

RESULTS

Production and Selection of α4β7 mAbs

Our immunization strategy led to the generation of more than 150 Ig-secreting immortalized hybridomas. In the first instance, 44 clones producing mAb able to bind to α4β7, based on indirect IIF of HEK cells transiently transfected with α4, β7, and/or α4β7, were selected (23 cells with a mixed β7/α4β7 profile, 14 cells with a mixed α4/α4β7 profile, 2 anti-α4 cells, and 5 anti-α4β7 cells). Next, we confirmed the specificity of 30 clones by flow cytometry of RPMI 8866, HEK-α4, HEK-β7, or HEK-α4β7 transfected cells. Assessment of the ability of the clones to block gp120 binding to α4β7 identified 7 clones that were able to strongly inhibit gp120/α4β7 interaction in vitro. Lastly, we selected 5 different mAbs, and these were named based on their respective isotypes, ie, DDX1430 (IgG1), DDX1431 (IgG1), DDX1435 (IgG1), DDX1432 (IgM), and DDX1434 (IgA).

The New mAbs Are Able to Recognize the Native Conformation of α4β7 Integrin

The newly generated mAbs were compared with well-characterized antibodies, such as Act-1 (anti-α4β7), FIB504 (anti-β7), 2B4 (anti-α4), and 8D3 (anti-β7).6,17,23 First of all, we compared their capacity to bind to RPMI 8866 cells, using flow cytometry. At a concentration of 5 μg/mL, DDX1430, DDX1432, DDX1434, DDX1435, Act-1, and 2B4 stained 95.2% ± 0.8%, 94.0% ± 0.7%, 99.3% ± 0.3%,
79.0% ± 2.5%, 82.4% ± 2.9%, and 98.1% ± 0.5% of the RPMI 8866 cells, respectively. Similar to FIB504 (which stained 65.3% ± 0.9% of the cells), DDX1431 only stained 57.34% ± 0.81% of the cells (Fig. 1A). The gating and flow cytometry strategies to assess β7 expression on CD45RO+ CD4+ T cells and binding of the different mAbs are shown in Supplemental Digital Content, Figure 1, http://links.lww.com/QAI/A973. We confirmed the higher binding capacity of the mAbs, relative to FIB504, on human α4β7⁺ CD45RO⁺ CD4⁺ T cells activated by anti-CD3, IL-2, and RA (Fig. 1B). To determine α4, β7, or α4β7 specificity, the purified mAbs were tested by flow cytometry (Fig. 1C) or IIF (Fig. 1D) on α4-, β7-, or α4β7-transfected HEK cells. All the clones were able to recognize α4β7-transfected cells at a concentration of 5 μg/mL, which was similar to FIB504, and better than Act-1. DDX1431 stained only 10.9% of the α4β7-HEK cells, although the other anti-α4β7 mAbs DDX1430, DDX1432, DDX1434, and DDX1435 recognized about 40% of the α4β7-transfected HEK cells (Fig. 1C). DDX1434 was the only one that could bind to α4-HEK cells, with a significantly higher binding capacity than the 2B4 mAb.
control (37% vs. 23%; \( P < 0.05 \)). DDX1434 was not able to bind to \( \beta^7 \)-transfected HEK cells, in contrast to DDX1430, DDX1431, DDX1432, and DDX1435 (which bound to 58.6%, 31.2%, 58.7%, and 56.1% of the cells, respectively) (Fig. 1C). No staining of nontransfected cells (HEK NT) was observed (Fig. 2C). In agreement with previous results, indirect IIF showed that DDX1430, DDX1431, DDX1432, and DDX1435 bound to \( \alpha^4 \)-transfected HEK cells (Fig. 1C). The entire panel of new mAbs specifically stained \( \alpha^4 \beta^7 \)-HEK cells, with a higher fluorescence intensity than what was detected for Act-1 and 2B4.

**The Newly Generated mAbs Have Higher Affinities**

First of all, we used enzyme-linked immunosorbent assay to assess the capacity of the mAbs to bind to the \( \beta^7 \) subunit (eg, \( \alpha^E \beta^7 \) and \( \alpha^4 \beta^7 \)) (Fig. 2A). As expected,
DDX1430, DDX1431, DDX1432, and DDX1435 bound to αEβ7 and α4β7 integrins, thus confirming their specificity for β7. DDX1435 had the highest affinity toward αEβ7 integrin. The mAbs had heterogenous affinities for α4β7 and αEβ7 (between 0.2 and 4 μg/mL for optimal binding), although these were all significantly higher than Act-1 (up to 10 μg/mL). The α4-specificity of DDX1434 (IgA) was also confirmed with α4β1 integrin (data not shown), with a profile that more closely matched that of anti-2B4.

Affinities of the clones and Kd and Ka constants were measured by SPR. The anti-β7-specific mAbs (ie, DDX1430, DDX1431, DDX1432, and DDX1435), and Act-1, recognized only α4β7 integrin, while not recognizing α4β1 and α6β4 integrins (Fig. 2B). The association (ka) and dissociation (kd) rate constants were determined by monitoring the change in SPR over time (Fig. 2C). The new IgA, IgM, and IgG1 mAbs had significantly higher Kds than did Act-1. Of note, DDX1435 (IgG1) had the highest affinity for α4β7. We also confirmed that DDX1434 (IgA) only targets α4 subunits on both α4β7 and α4β1 integrins (Kd = 4.45 10^{-10} and Kd = 4.32 10^{-12}).

**FIGURE 3.** Assessment of the mAb binding and blocking on memory CD4^+ T cells. Inhibition of gp120 (A) and MAdCAM-1 (C) binding on RA-treated memory α4β7^+ CD4^+ T cells by the various anti-α4β7 mAbs is shown on flow cytometry dot plots. Control anti-α4 mAb (2b4), anti-MAdCAM-1, and anti-CD4 (leu3a) were also used. The median IIF intensity (MFI) values are grouped in bar graphs (B and D). The statistical comparison between no gp120/leu3A and MAdCAM-1 binding to anti-α4β7 mAb was performed by 2-way analysis of variance. P-values less than 0.1 (*), 0.01 (**), 0.001 (***) and 0.0001 (****) are marked by asterisks.
FIGURE 4. The new α4β7-specific mAbs delay HIV replication. The capacity of each mAb to inhibit HIV replication in RA-treated α4β7+CD4+ T cells was evaluated by measuring the p24 concentration in culture supernatants. CD4+ T cells were preincubated with the anti-α4β7 mAbs before adding either SF162 HIV strain subtype alone, or coupled to RPMI 8866 cells. 92US660 and 92UG029 strains were also tested on their own. The statistical comparison between no mAb and anti-α4β7 mAb was performed by 2-way analysis of variance. P-values less than 0.1 (*), 0.01 (**), 0.001 (***) and 0.0001 (****) are marked by asterisks.

The mAbs Effectively Block gp120-α4β7 and MAdCAM-1-α4β7 Interactions

Using flow cytometry, we next assessed the ability of the mAbs to block gp120 (purified from CHO-SEC and Cap88) and MAdCAM-1 binding to α4β7 integrin on RPMI 8866 cells (data not shown) and α4β7hi memory CD4+ T cells (Figs. 3A, B, C, D). RA-activated CD4+ T cells were first incubated with blocking anti-CD4 mAb (Leu3a), as described previously. The binding of biotin-labeled gp120 and/or MAdCAM-1 was not altered by the IgG1 or IgM controls. Interestingly, DDX1434 (IgA) very efficiently and significantly inhibited both gp120 (Fig. 3B) and MAdCAM-1 (Fig. 3D) binding to α4β7. Although DDX1434 blocked gp120 binding to α4β7-specific IgA at levels similar to 2B4, its capacity to block MAdCAM-1 binding was significantly higher than that of 2B4 (92% vs. 77.7%).

The New α4β7-specific mAbs Interfere With HIV Infection

We performed in vitro HIV infections with 3 different primary strains so as to compare the ability of the mAbs to reduce and/or delay HIV infection of RA-activated α4β7hi CD4+ T cells. At d6, 2B4 (at 0.625 μg/mL) decreased SF162 but not 92UG029 and 92US660 infections. By contrast, DDX1431 (IgM), DDX1430, and DDX1432 (IgG1) amplified the infection by all the HIV strains that were tested (Fig. 4). As for Act-1, DDX1435 (IgG1) reduced SF162 and 92UG029 infections at d6. DDX1434 (IgA) decreased SF162 and 92UG660 infections, but not 92UG029 infection. DDX1431 (IgM) was able to delay infection by the 92UG660 strain only.

By using RPMI 8866 cells coated with SF162 particles followed by transfer to RA-treated CD4+ T cells, we were able to use a low virus dose, as described previously.12 We first verified that the RPMI 8866 cells were not infected by SF162 virus at d12 (data not shown). SF162 infection of RA-activated CD4+ T cells was significantly decreased by DDX1434 (IgA) and DDX1435 (IgG1) at d3 and d6 postinfection, as was also the case with control Act-1, 2B4, and DDX1431 (IgM) at d3. Pretreatment with these mAbs decreased p24 levels by more than 50%. No effect was seen with the other IgG1s at d3 and 6.

The New mAbs Recognize Both Human and Mouse α4β7+ T Cells on Human and Mouse Inflamed Biopsies

The ability of the mAbs to detect α4β7 integrin was measured by immunohistochemistry of human and mouse inflamed gut biopsies. The clones stained mouse and human tissue-restricted α4β7+ T cells, as did Act-1 did (Figs. 5A, B). However, DDX1431 appears to be less potent than other clones. DDX1430, DDX1432, DDX1435 (IgG1), and DDX1431 (IgM) were also able to detect intraepithelial cells that express αεβ7 in inflamed gut tissues (villi). DDX1434 (IgA) also stained α4β1+ mucosal cells because of its specificity for the α4 subunit. Binding of the mAbs was more pronounced on the intestinal villi, such as tertiary lymphoid tissues, which contained an autoreactivity/polyreactivity of the mAbs was determined with human epithelial type 2 cells (data not shown).
DISCUSSION
Lymphocyte recruitment into gastrointestinal tracts involves a multitude of molecules, such as selectins, integrins, and chemokines. Integrin α4β7 is a pivotal receptor for lymphocyte homing to GALT and has recently been described as a targeting receptor for HIV. Both IBD and HIV generate deleterious intestinal inflammation caused by massive lymphocyte infiltration, resulting in microbial translocation, malabsorption, and intestinal disorders. Targeting of α4β7 integrin by mAbs improves clinical scores in CD and ulcerative colitis, although optimization of current therapies will be required to reduce adverse side effects. Since the early

FIGURE 5. Ability of the various mAbs to identify α4β7+-expressing lymphocytes in mouse and human inflamed mucosal tissues by indirect IIF microscopy. The same concentration of mAbs (1 μg/mL) was used to determine the ability of the mAbs to stain lymphocytes on cryosections of Peyer’s patches from patients with dextran sodium sulfate–induced mouse colitis (A) or from patients with CD (B). FITC, fluorescein isothiocyanate.
1990s, several mAbs against α4, β7, and α4β7 have been developed in the mouse or rat, and some of these have been humanized and have undergone testing in human trials. In light of the role of this integrin during infection, anti-α4β7 mAbs could be used for HIV treatment, as they may counteract HIV GALT trafficking, and/or reduce potential HIV-specific α4β7+ target cells in the gut that sustain HIV replication. Natalizumab, derived from the HP2/1 mouse antibody, is an IgG4 that targets the α4 subunit, and it is able to reduce lymphocyte recruitment into the GALT and into the central nervous system by blocking MadCAM-1, VCAM-1, fibronectin, and osteopontin binding to αβ1 and α4β7. However, the ensuing immunodeficiency in the brain tissue induces JC virus reactivation. To avoid this deleterious effect, other mAbs that target either β7 or α4β7 have been developed that should reduce cell infiltration into GALT but not into the central nervous system. Anti-α4β7 mAbs have been demonstrated to have potential for treating IBD. Mouse Act-1 (vedolizumab is the humanized form of Act-1), mouse HP2/1 (natalizumab), and rat FIBS04 (etorizumab) were generated by immunization with an “empirical cell line,” in which α4β7 expression is not well defined. To generate α4β7 mAbs with high affinities and specificities, we used 3 different forms of α4β7 immunogens to mimic the native folding of the integrin. However, most of the produced mAbs were specific for β7 because of its higher immunogenicity. Of 150 hybridomas, we selected the clones (IgG, IgA, and IgM) with a high specificity to α4β7 and a high blocking ability for gp120 and/or MadCAM-1 interaction with α4β7. We ultimately focused on 4 β7-specific mAbs (3 IgGs and 1 IgM) and 1 α4-specific mAb (1 IgA), as they strongly recognized native conformation cell-expressed α4β7 (on RPMI 8866 cells, RA-treated human CD4+ T cells, and HEK transfected cells). By SPR, DDX1435 IgG and DDX1434 IgA were found to display higher affinity to α4β7 than Act-1 and FIBS04. Moreover, these 2 DDX mAbs were also able to efficiently block gp120 binding and delay HIV replication, thus demonstrating that our immunization strategy is very efficient. However, DDX1435, which is not able to fully block gp120 binding, reduced and delayed HIV replication on RA-treated CD4+ T cells. High-affinity mAbs could block HIV-1 binding to α4β7 and/or prevent LFA-1 activation, which is involved in HIV spreading from cell to cell. We also observed that pretreatment with our IgM mAb reduced p24 production at d6 for the 92US660 strain only, thus demonstrating that the α4β7 receptor is not essential for replication of all HIV strains. At high concentrations of Act-1, HIV replication was increased, and this was probably by promotion of cell spreading. However, Act-1 is able to delay infection in SIV-infected monkeys. To mimic mucosal infection, where α4β7/gp120 interaction seems to promote exponential replication from founder virus, a good in vitro predictive model could be of use. To this end, we transfected HIV precoated RPMI 8866 cells to RA-activated CD4+ T cells pretreated with anti-α4β7 mAbs. Act-1, 2B4, DDX1435, and DDX1434 were able to reduce and delay HIV replication in this experimental system. For DDX1434 IgA, which targets both α4β1 and α4β7, a titration of the mAb is required to investigate its potential side effects. However, mucosal administration of this IgA seems to be promising, and it may reduce the deleterious systemic effects observed with intravenous natalizumab injection. DDX1431 IgM has a low affinity for β7 and α4β7, although it was able to stain α4β7+ cells in gut inflamed tissues, and it could hence be an attractive molecule for mucosal application. Only DDX1434 IgA inhibited gp120 and MadCAM-1 binding. Further investigations are needed to evaluate the in vivo ability of this mAb to reduce gut inflammation in an animal model of colitis. Moreover, we previously reported that IgAs are also able to mediate reverse transcytosis after M-cell binding in Peyer’s patches, which could be very relevant for oral administration of α4β7-specific mAbs. A future evaluation of the capacity of DDX1434 IgA to block the interaction between α4β7 and VCAM-1 could expand its potential utility to asthma treatment by reducing recruitment of proinflammatory cells to the lung. Macaques treated very early after SIV infection with natalizumab exhibit a decrease in SIV DNA in the brain, and this is presumably due to blocking of trafficking and accumulation of SIV-infected monocytes/macrophages in the brain.11,30 Anti-α4β7 IgG mAb treatment of SIV infection also reduces transmission and SIV DNA in brain tissues and in GALT by blocking immune cell trafficking.30,31 The activity of our mAbs should be evaluated both by mucosal or by systemic administration before or during SIV challenge, and for IBD treatment. The potency of the anti-α4β7 IgA could also be evaluated, after oral or topical administration in the genital mucosa, as a way to counteract α4β7–HIV-1 interactions and to disrupt trafficking of α4β7–CD4+ T cells into GALT.

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