β₂ Integrin Is the Major Contributor to the αβ
Integrin-Mediated Blockade of HIV-1
Replication

Ester Ballana, Eduardo Pauls, Bonaventura Clotet, Françoise
Perron-Sierra, Gordon C. Tucker and José A. Esté

J Immunol 2011; 186:464-470; Prepublished online 22
November 2010;
doi: 10.4049/jimmunol.1002693
http://www.jimmunol.org/content/186/1/464

Supplementary Material
http://www.jimmunol.org/content/suppl/2010/11/19/jimmunol.1002693.DC1

References
This article cites 40 articles, 16 of which you can access for free at:
http://www.jimmunol.org/content/186/1/464.full#ref-list-1

Subscription
Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
β5 Integrin Is the Major Contributor to the αv
Integrin-Mediated Blockade of HIV-1 Replication

Ester Ballana,* Eduardo Pauls,* Bonaventura Clotet,* Françoise Perron-Sierra,†‡
Gordon C. Tucker,†‡ and José A. Esté*

Monocytes and macrophages are targets of HIV-1 infection and play critical roles in multiple aspects of viral pathogenesis. During the differentiation of monocytes to macrophages, adhesion molecules such as integrins are upregulated; therefore, they provide signals that control the process and subsequently may render macrophages more susceptible to HIV-1 infection. Previous work demonstrated that blocking αv-containing integrins triggered a signal transduction pathway leading to the inhibition of NF-κB–dependent HIV-1 transcription. In this paper, we show the influence of the different αv-coupled β integrins in HIV-1 replication in macrophages. Inhibition of β integrins, either by specific mAbs, small arginine-glycine-aspartic acid (RGD) mimetic compounds, or RNA interference, showed that integrin β5 was the major contributor to the integrin-mediated blockade of HIV-1 replication. Importantly, such inhibition did not induce changes in cell adhesion to the substrate. In conclusion, our results reveal a significant role of the integrin dimmer αvβ5 in HIV-1 infection of macrophages. *The Journal of Immunology, 2011, 186: 464–470.

Tissue macrophages play a central role in immune response pathways and are major targets for chronic infection by viruses including HIV-1. Infected macrophages constitute a stable viral reservoir that facilitates spread of HIV-1 to other cells or tissue compartments and contribute to immune pathogenesis (1, 2). Monocytes, for their part, are much less permissive to infection with HIV, both in vitro and in vivo, where estimates of infected circulating monocytes are consistently low (reviewed in Ref. 3).

The precise external signals that control differentiation of peripheral blood monocytes to tissue macrophages are incompletely defined. Adhesion molecules participating in the firm arrest and diapedesis of blood monocytes across endothelial and extracellular matrix barriers could provide signals that control this differentiation and subsequently may render macrophages more susceptible to HIV-1 infection. In addition, it has been shown that the monocyte to macrophage differentiation process entails an upregulation of integrin expression (4–6).

Integrins are a family of transmembrane receptors that mediate adhesion of cells to extracellular matrices, as well as intercellular interactions. These interactions transduce signals that control complex cell functions such as proliferation, differentiation, and survival, and require the regulation of gene expression (7). Different enveloped and nonenveloped viruses use integrins to enter and replicate in host cells (reviewed in Ref. 8). Integrins may serve as viral receptors, mediating virus attachment to the cell and generating signaling responses that promote viral replication (8–10).

We previously identified a possible role of αv-containing integrins in HIV infection of macrophages by demonstrating the antiviral effect of specific anti-αv Abs, as well as an arginine-glycine-aspartic acid (RGD) mimetic compound selective for αvβ3 and αvβ5 integrins (10, 11). We showed that inhibition or blockade of αv-containing integrins triggered a signal transduction pathway leading to downregulation of NF-κB–dependent HIV-1 transcription. Such inhibition was mediated by a MAPK signaling cascade, revealing a significant role of αv integrin-mediated adhesion and signaling in HIV-1 infection of macrophages (10).

All integrins are heterodimers composed of noncovalently linked α and β subunits. In humans, at least 18 α and 8 β subunits have been identified, forming more than 20 heterodimers, each of them with different cell expression patterns and ligand specificities (12). Integrin αv can be coupled with various β integrin subunits (β1, β3, β5, β6, and β8), forming heterodimeric proteins that could differently affect the antiviral activity reported earlier. However, little is known regarding the contribution of β integrins in HIV infection. In this study, we analyzed the role of the β-integrin component in αv-integrin heterodimers when primary monocyte-derived macrophages (MDMs) are challenged with HIV-1. Our results suggest a preferential implication of β5 integrin in HIV-1 infection of MDMs.

Materials and Methods

Cells

PBMCs were obtained from blood of healthy donors using a Ficoll-Paque density gradient centrifugation. To obtain the monocyte population, we blocked 300 million PBMCs with an anti-CD32 Ab (StemCell Technologies, Vancouver, British Columbia, Canada) and separated monocytes by a negative selection Ab mixture (StemCell Technologies) supplemented with an anti-CD41 Ab (StemCell Technologies) to eliminate platelets. Cells from monocyte/macrophage lineage, identified by flow cytometry as CD14+, were obtained with a purity ≥85%. Monocytes were resuspended in complete culture medium: RPMI 1640 medium (Life Technologies, Madrid, Spain) supplemented with 10% heat-inactivated FCS, 100 U/ml penicillin, 100 μg/ml streptomycin, and monocyte CSF (PeproTech, London, U.K.) at 20 U/ml. Then monocytes were differentiated for 3 d at 50,000 cells/well in 96-well plates for viability and acute infection experiments.

Received for publication August 9, 2010. Accepted for publication October 24, 2010.

This work was supported by the Spanish Ministerio de Ciencia e Innovación (Projects BFU2009-06958, SAF2007-63622-C02, and FIPSE 360783-09). E.B. is a research fellow from Fondo de Investigación Sanitaria. Address correspondence and reprint requests to Dr. José A. Esté, IrsiCaixa, Hospital Universitari Germans Trias i Pujol, Carretera Del Canyet s/n, Badalona, Barcelona 08916, Spain. E-mail address: jaeste@irsicaixa.es

Copyright © 2010 by The American Association of Immunologists, Inc. 0022-1767/10S16.00

www.jimmunol.org/cgi/doi/10.4049/jimmunol.1002693

The Journal of Immunology
**Drugs and Abs**

S36578 (5-(5S)-7-[(4-(pyridin-2-ylamino)-butyrylamino)-methyl]-6,9-dihydro-5H-benzocyclohepten-5-yl) acetic acid [compound 1.2 synthesized as described by Perron-Sierra et al. (13)] is a selective small nonpeptide heterocyclic RGD-mimetic compound targeting α5β1 and α6β4 integrins (13–15). S36578 analogs were obtained from Institut de Recherches Servier (Croissy sur Seine, France) (13). The reverse transcriptase (RT) inhibitor 3-azido-3-deoxythymidine (zidovudine [AZT]) was purchased from Sigma-Aldrich (St. Louis, MO), and the HIV replication, whereas no effect was observed when using an isotype control or Abs against integrins β1, β5, and β6. Percentage of HIV-1 BaL replication measured as p24 in the supernatant 7 d post-infection. Mean ± SD of three independent experiments is shown. All concentrations are expressed in μg/ml. *p < 0.05; **p < 0.005; ***p < 0.0005. Cap24, capsid p24 Ag; ND, no drug.

**Flow cytometry**

MDMs were detached with trypsin-free commercial EDTA/PBS solution (Versene; Invitrogen, Paisley, U.K.) and, before staining, incubated for 20 min with human IgG at 1 mg/ml. Cells were then stained with the following mAb: α5-PE (Santa Cruz Technologies, Santa Cruz, CA), β1-

**FIGURE 1.** β-integrin expression in MDMs. A. Representative flow cytometry histograms (top panels) showing expression of CD14 and integrin α5 in MDMs 3 d postdifferentiation. Representative flow cytometry histograms (bottom panels) showing expression of integrins β1, β3, and β5. Expression of integrins β6 and β8 could not be detected. A representative experiment is shown. B. Relative integrin mRNA expression quantified by real-time PCR. Bars represent the difference in amplification cycle between the corresponding β integrin and the control GAPDH gene run in parallel; that is, the higher the bar, the less mRNA. β6 integrin data are missing because mRNA levels were under the detection level of the technique. Mean ± SD of three independent donors is shown.

**FIGURE 2.** Inhibition of HIV-1 replication in the presence of β-integrin Abs. Blockade of integrins β3 and β5 impaired HIV replication, whereas no effect was observed when using an isotype control or Abs against integrins β1, β6, and β8. Percentage of HIV-1 BaL replication measured as p24 production in the supernatant 7 d post-infection. Mean ± SD of three independent experiments is shown. All concentrations are expressed in μg/ml. *p < 0.05; **p < 0.005; ***p < 0.0005. Cap24, capsid p24 Ag; ND, no drug.

**Immunofluorescence**

Cells were grown and differentiated on coverslips (multichambered coverslips; Invitrogen) before fixation with formaldehyde 1%. Fixed cells were rinsed with PBS, blocked and permeabilized with 0.01% Triton X-100, 5% FCS in PBS for 1 h at room temperature. Afterward, cells were incubated overnight at 4°C with mouse anti-human β integrin Abs (β3 1/50 [Novus Biologicals]; β5 1/50 [Abnova]) diluted in PBS with 0.5% FCS. The primary Ab was detected by incubation with an anti-mouse secondary biotin-conjugated Ab at room temperature for 1 h (1:200; BD Biosciences) followed by incubation with streptavidin-FITC (1:200; SBA, Birmingham, AL) and cultures were counterstained for F-actin with rhodamine-conjugated phalloidin (1 μg/ml; Sigma-Aldrich) for 30 min. Cells were then rinsed and mounted with Prolong Gold antifade reagent (Molecular Probes, Eugene, OR; Invitrogen). Cell preparations were viewed with an Olympus PV 1000 Confocal Microscope (Olympus, Barcelona, Spain).

**Quantitative RT-PCR**

Total cellular RNA was purified from transfected cells by RNasy mini kit (Qiagen, West Sussex, U.K.), as recommended by the manufacturer, including the DNase I treatment step. mRNA relative levels of α5, β1, β3, β6, and β8 integrin subunits were measured by two-step quantitative RT-PCR and normalized by comparative threshold cycle method to GAPDH.
mRNA expression. In all experiments, a sample treated in the absence of RT enzyme was used as a negative control. Primers and DNA probes were purchased from Applied Biosystems (assay-on-demand; Applied Biosystems, Madrid, Spain), selecting assays whose probe span an exon junction to avoid detecting genomic DNA.

Small interfering RNA transfections

Small interfering RNAs (siRNAs) were transfected using the Amxa Nucleofection technology (Lonza, Basel, Switzerland) as described previously (10, 23). In brief, 10^6 freshly isolated monocytes were transfected with 200 nM of the corresponding siRNA using the human monocyte nucleofector kit (Amxa) and following manufacturer’s recommendations. After nucleofection, cells were recovered and seeded in prewarmed 24-well plates in Human Monocyte Nucleofector medium (Amxa) for 12 h. The day after transfection, cells were recovered and differentiated by adding monocyte CSF to the medium, as described earlier.

All siRNAs targeting the different β integrins, as well as the control nontargeting siRNAs, were commercially available pools of four different siRNAs (Dharmacon Smartpools, Thermo Scientific, Madrid, Spain).

HIV infection and replication

The R5 HIV-1 strain BaL was grown in stimulated PBMCs. MDMs were infected with the R5 HIV-1 strain BaL on day 3 after monocyte CSF stimulation as described previously (10, 24). In all cases, compounds were added simultaneously with the virus. Every 3 d, 10% culture supernatant was replaced by 100 μl culture supernatant supplemented, fresh complete medium with or without the corresponding drug or Ab. HIV production was analyzed 7 or 10 d postinfection by ELISA HIV-p24 Ag detection in culture supernatants (Innogenetics, Barcelona, Spain).

Viability assays

For cell viability, MDMs were transfected or treated with the same drugs at the same concentrations as for acute infection experiments. Measurement of cell cytotoxicity was performed by a methyl tetrazolium-based colorimetric assay (MTT method) (25, 26).

Statistical analysis

Data are presented as mean ± SD. Paired Student t test was used for comparison between two groups, using the GraphPad Prism software (GraphPad, San Diego, CA). The p values <0.05 were considered significant.

Results

Differential expression of α,β-coupled β integrins in macrophages

MDMs differentiated for 3 d with monocyte CSF were examined for the expression of the different β-integrin subunits that can interact with the α, subunit to form a functional αβ heterodimeric integrin.

Almost all MDMs expressed α6 (97 ± 2% positive cells) and β1 (98 ± 1% integrins), whereas β2 (83 ± 2%) and β3 (50 ± 18%) subunit expressions were more heterogeneous, as seen by flow cytometry (Fig. 1A). The expression of other β-integrin subunits known to be coupled with α6, β3, and β4 were not detected by flow cytometry in MDMs (data not shown). Quantitative PCR data also showed low or undetectable levels of mRNA expression of β3 and β6 subunits, thus suggesting that both are little or not expressed in MDMs (Fig. 1B and data not shown).

Integrin β1 is known to be highly expressed in undifferentiated monocytes (6), whereas α6, β3, and β4 integrins are upregulated during the differentiation process from monocytes to macrophages (5, 6, 11), the latter being, therefore, the most plausible candidates to influence HIV infection outcome.

Blockade of integrins β2 and β3 by Abs inhibited HIV-1 replication in macrophages

To evaluate the effect of α,β-coupled β integrins on HIV replication, mAbs targeting the different β integrins were selected. MDMs were treated with anti-β-integrin Abs at the time of infection with HIV-1 BaL strain, and viral replication was measured 7 d later. Cell toxicity was controlled in uninfected cultures performed in parallel and measured by MTT colorimetric assay. None of the Abs used decreased cell viability (data not shown).

As expected, treatment with the HIV-1 RT inhibitor AZT or with the CCR5 antagonist TAK-779 potently blocked HIV-1 replication (99.8 ± 0.8% and 82 ± 7% inhibition compared with control MDMs, respectively), whereas the RGD mimetic compound S36578 partially blocked HIV replication (60 ± 14% inhibition compared with control MDMs at the greater concentration tested; Fig. 2). The mAbs targeting β1, β4, and β3 integrins did not significantly inhibit HIV-1 replication, nor did the mouse IgG1 Ab that was used as an isotype control (8 ± 18% inhibition for the isotype control; 17 ± 35% increase for β1; 21 ± 20% inhibition for β4 and 5 ± 50% inhibition for β3 mAbs, compared with control MDMs and at the greater concentrations tested for each mAb).

Interestingly, both β1 and β6 mAbs were able to partially block viral replication in MDMs (51 ± 13% and 53 ± 11% inhibition compared with control MDMs for the greater concentrations tested of β1 and β6 mAbs, respectively; Fig. 2). Inhibition was dose-dependent with a calculated EC_{50} of ~0.94 μg/ml for both β1 and β6 mAbs. These results pointed to β1 and β3 integrins as the key partners in the α,β-dependent blockade of HIV replication.

<table>
<thead>
<tr>
<th>Compound</th>
<th>EC_{50} (μg/ml)</th>
<th>α6,β1</th>
<th>α6,β3</th>
</tr>
</thead>
<tbody>
<tr>
<td>S36578</td>
<td>0.72</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>C1</td>
<td>0.43</td>
<td>0.7</td>
<td>0.5</td>
</tr>
<tr>
<td>C2</td>
<td>0.17</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>C3</td>
<td>0.28</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>C4</td>
<td>0.24</td>
<td>0.6</td>
<td>0.5</td>
</tr>
<tr>
<td>C5</td>
<td>1.62</td>
<td>73</td>
<td>10</td>
</tr>
<tr>
<td>C6</td>
<td>2.07</td>
<td>4</td>
<td>1.4</td>
</tr>
<tr>
<td>C7</td>
<td>2.18</td>
<td>38</td>
<td>40</td>
</tr>
<tr>
<td>C8a</td>
<td>&gt;5</td>
<td>3011</td>
<td>1276</td>
</tr>
</tbody>
</table>

A relation between the antiviral activity and the integrin affinity in binding assays is clearly shown.

α6,β3 or low m.w. antagonists.

Table I. Inhibition of HIV-1 replication in the presence of α6,β3 and α6,β4 low m.w. antagonists.

FIGURE 3. Correlation between the antiviral potency and the affinity to bind integrins in vitro observed of α6,β3 and α6,β4 low m.w. antagonists. Linear regression analysis of EC_{50} anti-HIV activity (x-axis) and IC_{50} integrin binding values (y-axis) for α6,β3 dimmer (dashed line) and α6,β4 (solid line). The correlation observed is an indication of the specificity of the blockade of HIV replication mediated by α6-containing integrins.
Antiviral activity of heterocyclic nonpeptide RGD mimetic compounds selective for \( \alpha_v\beta_3 \) and \( \alpha_v\beta_5 \) integrins

To further confirm and investigate the role of \( \beta \) integrins 3 and 5, we evaluated a group of heterocyclic nonpeptide RGD mimetic compounds with different selectivities for \( \alpha_v\beta_3 \) and \( \alpha_v\beta_5 \) integrins (13) for their capacity to inhibit HIV-1 replication in MDMs. The compounds were added to differentiated MDMs at the time of viral infection and HIV replication was monitored after 7 d by measuring production of capsid Ag p24 in the culture supernatant.

The compounds tested comprised three structurally unrelated potent inhibitors of \( \alpha_v\beta_3 \) and \( \alpha_v\beta_5 \) integrins (S36578 and compounds C1 and C3), together with five inhibitors related to S36578 (compounds C2 and C4 to C7) identified during structure-activity studies, with various potencies (13). They were all able to block HIV replication with different efficacies, depending on their affinity toward the corresponding integrins in binding assays (Table I). We also evaluated the heterocyclic building block of S36578 lacking the selectivity- and potency-conferring crucial arginine mimetic moiety (compound C8) and found no activity on HIV replication up to 5 \( \mu \)g/ml. The correlations between the antiviral potency and the affinity to bind integrins in vitro observed with these compounds (\( R^2 = 0.77 \) and 0.74, respectively, for \( \beta_3 \) and \( \beta_5 \) integrins) is an indication of the specificity of the blockade of HIV replication mediated by \( \alpha_v \)-containing integrins, and specifically by the dimmers formed with \( \beta \) subunits 3 or 5 (Fig. 3). In addition, it provides further evidence of the involvement of \( \beta_3 \) and \( \beta_5 \) integrins in HIV replication of MDMs.

Inhibition of \( \beta_v \) integrin expression by siRNA impaired HIV-1 replication in macrophages

To further confirm the role of the different \( \beta \) integrins as contributors in HIV replication in MDMs, we used different siRNAs specifically targeting each \( \alpha_v \)-coupled \( \beta \)-integrin subunit. A non-targeting siRNA pool was used as a control. Because the \( \beta_6 \) subunit integrin has undetectable expression in MDMs, siRNAs against it were also used as a control.

Efficient and specific inhibition of the different \( \beta \) integrins (except for \( \beta_6 \), see earlier) was achieved by RNA interference both at the mRNA (Fig. 4A) and the protein level (Fig. 4B, 4C) for integrin \( \beta_1 \) (91 \( \pm \) 1\% downregulation), \( \beta_3 \) (70 \( \pm \) 15\% downregulation), and \( \beta_5 \) (90 \( \pm \) 5\% downregulation), respectively, in cell surface protein expression compared with mock-treated cells. Conversely, the siRNAs used did not inhibit \( \beta_6 \)-integrin expression, either because the siRNA pool lacked potency or, more probably, because of the reduced expression of \( \beta_6 \) in macrophages, which made it difficult to detect. Inhibition of \( \beta \)-integrin subunits by siRNA led to downregulation of cell surface expression of both \( \beta \) and its \( \alpha \) counterpart in MDMs (Fig. 4B, Supplemental Fig. 1) as reported in HeLa-MAGI cells (10). The specific inhibition of \( \beta \)-integrin subunits did not induce a downregulation of the \( \alpha_v \)-integrin subunit expression at mRNA level (Fig. 4A). These results suggest an impairment of the \( \alpha_v \) dimmer formation, because of the inhibition of the corresponding \( \beta \)-integrin partner.

Previous work showed that treatment of MDMs with the RGD mimetic compound S36578 led to a change in cell morphology that,
although not affecting cell viability, unequivocally affected cell adhesion to the substrate (10). In this work, no changes in cell morphology or cell adhesion to the substrate were observed as a result of downregulation of the β5-integrin subunits (Fig. 5), suggesting that αv-mediated adhesion is not affected when a single β subunit is inhibited.

Acute infection of siRNA-treated cells with BaL HIV-1 strain showed that only β3 downregulation inhibited HIV-1 replication (44 ± 11% inhibition for siβ3) compared with mock-treated cells or cells treated with a control siRNA (112 ± 10% replication; Fig. 6). No changes in HIV replicative capacity were observed as a result of the downregulation of the other β-integrin subunits, pointing to the α5β3 heterodimer as the major contributor to the integrin-mediated HIV blockade. The impairment in HIV replication because of β3 downregulation was similar but not as potent as that obtained with S36578 (71 ± 12% inhibition), suggesting that the blockade of αv subunit was also contributing, to some extent, to the antiviral effect observed. Treatment with AZT, an HIV-1 RT inhibitor, or the CCR5 antagonist TAK-779 potently blocked HIV-1 replication (99 ± 0.03% and 96 ± 4% inhibition for AZT and TAK-779, respectively). Treatment of MDMs with siRNA did not affect cell viability as measured by MTT colorimetric assay (data not shown).

![Figure 5](Image)

**FIGURE 5.** Downregulation of αv-coupled β integrins did not affect cell shape or adhesion. Immunofluorescence of MDMs mock-transfected or treated with siRNAs [nontargeting siRNA, siβ3 (A) and siβ5 (B)]. Cells were immunostained for the corresponding β integrin (green) and counterstained for F-actin with phalloidin (red). No changes in cell shape and adhesion were observed as a consequence of β-integrin downregulation.

![Figure 6](Image)

**FIGURE 6.** Inhibition of β3-integrin expression by siRNAs blocked HIV-1 replication in MDMs. siRNA downregulation of integrin β3 significantly inhibited HIV-1 BaL replication. Percentage of HIV-1 BaL replication measured as p24 production in the supernatant 9 d post-infection. Mean ± SD of three independent experiments is shown. *p < 0.05; **p < 0.005; ***p < 0.0005 compared with untreated cells. ND, no drug; siNT, nontargeting siRNA control.

**Discussion**

Monocytes/macrophages play a major role in HIV transmission throughout all stages of HIV infection and disease. Aberrant HIV-induced macrophage behavior includes a direct contribution to the establishment, spread, and persistence of the infection; as long-living primary target cells of HIV with a widespread dissemination and a persistent failure to enter apoptosis on infection, they represent an important cellular reservoir for the virus (1, 27, 28). Thus, a complete understanding of HIV-1 pathogenesis must incorporate an elucidation of genetic/molecular events induced by the virus in macrophages and how such changes modify the outcome of viral infection. Cell-to-cell contacts, cell adhesion to the substrate, and cell differentiation state enhance HIV replication (29–31). Adhesion molecules including integrins have been recognized as host factors that influence viral replication including HIV (6, 8, 10, 11, 32–34). Moreover, we and others have demonstrated a role of integrin αv in HIV pathogenesis in MDMs (6, 10, 11). Because integrins are functional as αβ heterodimers and αv interacts with several β subunits, we sought to determine which β-integrin subunits, by coupling to αv, affect HIV replication. In this article, we identified β3 integrin as the most important αv partner controlling HIV replication in MDMs.

The five β integrins that couple with the αv subunit are expressed in a variety of cells and tissues, and their expression is tightly regulated (7, 12). In our model of in vitro differentiated MDMs, only three of the β integrins (β1, β3, and β5) were expressed. One of them, integrin β3, was known to be already expressed in monocytes (6), therefore pointing to integrins β3 and β5 as the most plausible candidates to be involved in the gained susceptibility to HIV infection, as a result of the differentiation process from monocytes to macrophages. Further analysis using mAbs targeting the different β integrins and the use of RGD mimetic compounds with different affinity for αvβ3 and αvβ5 confirmed this first observation. A 56% identity was found between the β3 and β5 protein sequences, making them the most closely related of the integrin β subunits (12), however, individual integrins have unique ligand specificities (reviewed in Refs. 7, 8, 12). Finally, expression downregulation of the different β-subunit genes by RNA interference demonstrated the major contribution of β3 integrin in the blockade of HIV replication in MDMs, but not β5. The disparity in the contribution of β3 integrin when using Abs or RNA interference could be the result of Ab cross-reaction, because of the high similarity between β3 and β5 integrins, both as monomers or when forming a dimer together with αv. However, RNA interference has been recognized as a useful tool to spe-
cifically test the effect of a single gene/protein, thus allowing for clear distinguishing between $\beta_2$ and $\beta_3$ functions.

Cell surface expression of integrin requires the expression of both $\alpha$ and $\beta$ subunits (7). The lack of one (e.g., $\beta_3$ or $\beta_2$) may lead to a decrease in the surface expression of its counterpart (e.g., $\alpha_x$). However, whereas downregulation of the $\alpha_\beta$ integrin led to inhibition of virus replication, $\alpha_\beta$ downregulation did not, providing further proof that the $\alpha_\beta$ integrin is responsible for the inhibitory effect.

All $\alpha_x$ integrins mediate cell adhesion to various matrix proteins, including fibronectin, vitronectin, tenasin, osteopontin, and fibrinogen, by recognizing sites containing the tripeptide sequence RGD (7). Although the first described member of this subfamily, $\alpha_5\beta_3$, appears to be the only one of all the $\alpha_x$ integrins that have been reported to be more restrictive in their interactions with ligands. For example, $\alpha_5\beta_3$ and $\alpha_6\beta_4$ have been reported to be principally vitronectin receptors, and $\alpha_6\beta_4$ has been reported to principally bind fibronectin and, to a lesser extent, vitronectin and tenasin.

The deletion of individual genes by knockout in mice showed that particular integrins play critical roles in different functions, also supporting the idea of $\beta_1$ and $\beta_3$ having different roles in specific scenarios. Knockout of the gene for $\beta_1$ enhanced tumorgenesis and angiogenesis, enhanced wound healing, and enhanced inflammation and atherosclerosis, suggesting that $\beta_1$ normally suppresses these processes (35). On the other hand, the $\beta_2$ knockout mouse suggested an important role for $\beta_2$ in keratinocyte adhesion and migration on vitronectin, but suggested that functions of $\alpha_\beta_2$ in cutaneous wound healing or adenovirus infection can be functionally replaced by other receptors (36). Thus, it is not surprising that, in the case of HIV, the $\alpha_\beta_2$ dimmer would have a more prominent role than the others.

Interestingly, the silencing of a single $\beta$-integrin subunit did not induce adhesion defects in MDMs, contrary to that reported when $\alpha_\beta_2$ subunit was blocked (10). This adhesion impairment when $\alpha_\beta$ function is inhibited could be because of the combined effects of loss of multiple members of the $\alpha$ integrin subfamily, whereas other family members can functionally replace the loss of a single $\beta$ subunit. Accordingly, mice lacking the $\alpha_5$ subunit die during embryonic development or immediately after birth (37). Conversely, $\beta_2$ and $\beta_3$ null mice were born viable (35, 36), suggesting that loss of $\alpha_5\beta_2$ or $\alpha_5\beta_3$ is not, by itself, responsible for the defects in $\alpha_5$ null mice. The fact that, by targeting only $\alpha_5\beta_3$ integrin, the antiviral activity was maintained without affecting cell adhesion is extremely important from a therapeutic point of view. The secondary undesired effects of blocking all $\alpha\beta$-related functions would be too severe; on the contrary, specifically inhibiting a single heterodimer could potentially have fewer contraindications from a systemic point of view.

Our previous work demonstrated an effect on HIV-1 transcription of integrin-mediated adhesion in MDMs, a mechanism that might drive the antiviral effect observed when inhibiting $\beta_3$ integrin. It is known that integrin-triggered signals cause activation and nuclear translocation of MAPKs (38). However, different signal transduction pathways involving calcium mobilization, PI3K activation, and downstream phosphorylation of MAPKs have been shown to control HIV transcription and posttranscriptional events (39). Thus, blockade of $\beta_2$-integrin-mediated signaling could also trigger the inhibition of HIV-1 transcription, similar to that reported earlier for its $\alpha$-subunit partner (10). Further studies are warranted to shed light on the specific signal transduction pathways involved.

Defining the cellular factors and mechanisms involved in the regulation of HIV infection could be important for the long-term goal of eradicating viral reservoirs in infected patients (40). Integrins remain attractive drug targets for interfering with cell proliferation, migration, or tissue localization of inflammatory, angiogenic, and tumor cells. Thus, drugs targeting integrins could modulate specific ligand affinity and signaling, a situation that could be useful in controlling HIV-1 and other infectious diseases.

Acknowledgments

We thank the National Institutes of Health AIDS Reference Reagent Program for reagents.

Disclosures


References


Supplementary Figure 1. AlphaV integrin expression in siBeta-treated MDMs.

Flow cytometry histograms showing expression of integrin AlphaV in MDM knocked down for integrin Beta1, Beta3 and Beta5 (dark line). Mock-transfected (dark grey line), and siNT MDM (light grey line) are shown as controls. No significant differences in the percentage of alphaV positive cells were observed compared to mock-transfected or siNT control cells (88% in mock-transfected vs. 87% in siBeta1; 89% in mock-transfected vs. 84% in siBeta3 and 88% in mock-transfected cells vs. 79% in siBeta5 MDM).