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Inhibition of human immunodeficiency virus type 1 infection in macrophages by an alpha-v integrin blocking antibody

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Abstract

Macrophages are key cells for HIV infection and HIV spreading inside the organism. Macrophages cultured in vitro can be successfully infected after differentiation with cytokines such as macrophage colony stimulating factor (M-CSF). In the monocyte to macrophage differentiation process with M-CSF, αv-integrins are upregulated concomitantly with the capacity of HIV to generate a productive virus infection. In the present study we show that an anti-αv antibody, 17E6, inhibited HIV-1 infection of primary macrophages. The effect of 17E6 on HIV-1 BaL replication in acutely infected macrophages was dose-dependent, with a 50% effective concentration (EC_{50}) of 17 ± 2 μg/ml in the absence of cytotoxicity. Similarly, a monoclonal antibody targeting the αvβ6 integrin (14D9.F8) also inhibited HIV-1 BaL infection in this cell type. 17E6 reduced the detection of HIV-1 BaL proviral DNA in acutely infected macrophages, but was completely ineffective against HIV-1 BaL production in chronically infected macrophages, suggesting that 17E6 inhibited HIV infection at an early stage of the virus cycle. Finally, a small molecular weight antagonist of the αvβ6 integrin, EMD 409849, reduced HIV replication at subtoxic concentrations. Therefore, our results suggest that αv-containing integrins could play a role in HIV replication in macrophages and suggest that small-molecular-weight compounds might interfere with HIV replication in macrophages through the interaction with αv integrins.

Keywords: Macrophages; HIV-1; Integrins; alpha-v; Macrophage colony-stimulating factor; Anti-HIV agents

1. Introduction

HIV-infected patients with a good response to antiretroviral treatment commonly fail to maintain an undetectable viral load when treatment is interrupted. The latent HIV-1 infection in resting $CD4^+$ memory T lymphocytes cannot fully explain this observation (Chun et al., 2000; Zhu et al., 2002). Monocyte/macrophage lineage cells are known to be an important HIV reservoir. Once infected, monocyte-derived macrophages (MDM) survive and produce high amounts of viral particles (Aquaro et al., 2002; Pereira et al., 2003). To reduce this long-lived reservoir, it is important to understand the mechanism by which MDM become persistently infected, and to develop new tools and new drugs to block HIV infection and virus production in macrophages.

A number of host factors can alter the expression of HIV receptors and coreceptors (Llano et al., 2003) that, in turn, can modulate the attachment and entry of HIV particles into the cell (Ugolini et al., 1999), HIV replicative capacity (Armand-Ugon et al., 2003b) or its ability to infect cells by cell to cell transfer (Blanco et al., 2004). Integrins are a family of cell surface receptors that mediate cell interactions with the extracellular matrix and with other cells. Integrins are heterodimers composed of α and β subunits. At least, 8 different β subunits (β1–β8) and 18 different α subunits (α1–α11, αv, αD, αE, αL, αM, αX, αIIb) have been identified (Hynes, 2002). Each subunit is mostly extracellular and crosses the membrane with a short cytoplasmic domain (Hynes, 2002; Xiong et al., 2003). Integrins have been shown to play a crucial role in adenovirus infections (Baranowski et al., 2001; Hippenmeyer et al., 2002).

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When monocytes are stimulated to differentiate to macrophages by culturing for several days in the presence of macrophage colony stimulating factor (M-CSF) or granulocyte-macrophage colony stimulating factor (GM-CSF), an upregulation of αv-integrins occurs (De Nichilo and Burns, 1993). Freshly isolated monocytes cannot be readily infected with HIV, and for a successful infection, differentiation of monocytes to macrophages with human serum or cytokines, like M-CSF, is required (Kutza et al., 2000; Lafrenie et al., 2002). Therefore, we have studied the role of αv-integrins in HIV infection of primary MDM treated with M-CSF. Our results suggest an implication of αv-integrins in HIV-1 infection of monocyte-derived macrophages.

2. Materials and methods

2.1. Cells

Peripheral blood mononuclear cells (PBMC) were obtained from peripheral blood of healthy HIV negative donors using a Ficoll-Paque density gradient centrifugation. To obtain the monocyte population, 400 million PMBC were blocked with an anti-CD32 antibody (StemCell Technologies, Vancouver, Canada), and then monocytes were separated by a negative selection antibody cocktail (StemCell Technologies, Vancouver, Canada), supplemented with an anti-CD41 antibody (StemCell Technologies) to eliminate platelets. Cells from monocyte/macrophage lineage, identified by flow cytometry as $CD14^+$, were obtained with a purity ≥90%. Monocytes were resuspended in complete culture medium: RPMI 1640 medium (Gibco, Madrid, Spain) supplemented with 10% heat-inactivated fetal calf serum (Gibco, Madrid, Spain), 100 U/ml penicillin, 100 μg/ml streptomycin and M-CSF (Peprotech, London, UK) at 20 U/ml (100 ng/ml). Then, monocytes were cultured for 3 days at 50,000 cells/well in 96-well plates for viability, acute infection and entry experiments. For chronic HIV-1 infection and receptor expression experiments, monocytes were cultured at 200,000 cells/well in 24-well plates to allow for extensive washing of infectious supernatant and harvesting for receptor expression analyses.

2.2. Flow cytometry analysis

After 3 days of culture with M-CSF, MDM were treated with trypsin-free Versene (catalog number 15040-033, GibCO, Madrid, Spain) for 5 min, and were carefully harvested and resuspended in phosphate-buffered saline (PBS) at 500,000 cells/ml. Then MDM were incubated for 20 min with human immunoglobulin G (IgG) at 1 mg/ml. After this incubation MDM were extensively washed and incubated with the appropriate monoclonal antibody (mAb): anti-CD4-PerCP, anti-CCR5-PE, anti-CD14-APC (BD, Madrid, Spain) or, for integrin expression, with the monoclonal antibodies: 17E6 (anti-αv), and 14D9.F8 (anti-αvβ6) (Mitjans et al., 1995). A mouse anti-human IgG1 (eBioscience, Madrid, Spain) was used as isotype control. For integrin staining, cells were incubated with 10 μg/ml of the corresponding mAb for 20 min at room temperature followed by an incubation with a secondary goat anti-mouse antibody (BD, Madrid, Spain) conjugated to FITC. Finally, cells were analyzed in a FACScalibur flow cytometer (BD, Madrid, Spain) with the CELLQUEST software (BD).

To evaluate if 17E6 could change the cell surface receptor expression, MDM plated at 200,000 cells/well in 24 well plates were treated with 17E6 at 20 μg/ml. Three days after addition of 17E6, MDM were treated with Versene (Gibco, Madrid, Spain) for 5 min, gently harvested and resuspended in PBS for flow cytometry analysis of CD14, CD4 and CCR5 expression, as described above.

2.3. Antiviral activity

After 3 day stimulation with M-CSF, cells were washed and incubated in complete culture medium containing various anti-HIV drugs and antibodies: the gp41 inhibitor C-34 (Armand-Ugon et al., 2003a; Esté, 2003) at 2 μg/ml, the reverse transcriptase (RT) inhibitor azidothymidine (AZT) (Sigma, Madrid, Spain) at 0.2 μg/ml, the anti-αv antibody 17E6, the anti-αvβ6 antibody 14D9.F8, and the anti-β6 antibody 5C4 (all provided by Merck FQ, Barcelona, Spain) (Hynes, 2002; Mitjans et al., 1995). A mouse IgG1 antibody (eBiosciences) was used as isotype control. A low-molecular-weight compound directed against αvβ6 integrin, EMD 409849 (Merck FQ, Barcelona, Spain), was also tested. MDM were infected with the R5 HIV-1 strain BaL at a final concentration of 3700 pg/ml of HIV-p24 antigen. At day 3, 7, 10 and 14 post-infection, 20 μl of culture supernatant were replaced by 20 μl of fresh complete medium with or without the corresponding drug or antibody. HIV production was analyzed 7 and 14 days after infection by HIV p24 antigen detection in the culture supernatants (Innogenetics ELISA kit, Barcelona, Spain).

For the evaluation in chronically infected MDM, acutely infected MDM were carefully washed, fed with complete medium and analyzed for p24 antigen production every 4 days, starting from day 3 post-infection. We considered that a chronic infection was established when at least two consecutive measures showed stable virus production, normally obtained 15 days after infection. For antiviral activity, chronically infected MDM were washed twice to remove any virus present in the supernatants and were replenished with complete culture medium and the corresponding drug or antibody (C-34 at 2 μg/ml, AZT at 0,2 μg/ml, the HIV protease inhibitor ritonavir at 7 μg/ml, 17E6 at 40, 20 and 5 μg/ml and 14D9.F8 at 20 μg/ml) in duplicate wells. Four days after drug or antibody addition, p24 antigen production was evaluated.

2.4. HIV entry assays

To evaluate HIV entry, MDM treated with C-34 at 2 μg/ml, 17E6 at 40 μg/ml or 14D9.F8 at 20 μg/ml were exposed to HIV-1 BaL (37000 pg of p24 antigen/ml) for 18 h and, finally, proviral DNA production was evaluated. The HIV-1 BaL concentration used was 10-fold higher than in the acute infection, because in HIV entry assays the readout was at 18 h and the parameter

evaluated was proviral DNA. After virus challenge, supernatant was removed and MDM DNA was extracted with a QIAamp blood kit (Qiagen, Hilden, Germany) as described by the Manufacturer. Proviral DNA was amplified by quantitative real-time PCR in an ABI-7000 Sequence Detection System (PE Biosystem, England), using primers CCTAGCATTTCATCACGTGGC and TTCTTGAAGTACTCCGGATGCAG corresponding to the HIV-1 LTR and SYBR Green detection (Applied Biosystems) as described before (Armand-Ugon et al., 2003a). To normalize HIV copy values per cell, amplification of cellular RNAseP gene was performed (Applied Biosystems). A standard curve was generated using dilutions of persistently infected 8E5 cells (Folks et al., 1986).

2.5. Viability assays

For cell viability, MDM were treated with the same drugs and antibodies at the same concentrations as in acute infection and entry experiments. At day 3, 7, 10 and 14, 20 μl of culture supernatants were replaced by 20 μl of fresh complete culture medium with or without drugs and antibodies. At days 1, 7 and 14 after the addition of drugs and antibodies, measurement of cell cytotoxicity was performed by a methyl tetrazolium-based colorimetric assay (MTT method) (Armand-Ugon et al., 2005; Bergamini et al., 1992; Esté et al., 1999). Twenty microlitres of MTT solution (Sigma) were added to each well, and plates were incubated at 37 °C in a CO_2 incubator for 2 h. Then, 150 μl of supernatant were harvested, followed by addition of 150 μl of isopropanol:Triton X-100:HCl (37%) at a ratio of 5:0.3:0.03 (v/v). The absorbance at 550/620 nm was measured using a plate reader. For the evaluation of cell viability in chronic infection, uninfected MDM were cultured under the same conditions as infected MDM. Cell viability was measured by the MTT method.

2.6. Antiviral assay

Anti-HIV activity of mAb 17E6, mAb 14D9.F9, mAb 5C4 and the low-molecular-weight compound EMD 409849 was measured against HIV-1 NL4-3 strain in MT-4 cells by a tetrazolium-based colorimetric method (MTT method) (Armand-Ugon et al., 2003a; Bosch et al., 2005). Activity of these αv-targeting antibodies and EMD 409849 compound was also assayed in PBMC stimulated for 72 h with 4 μg/ml phytohemagglutinin (PHA) and 6 U/ml interleukin (IL)-2 and later infected with the R5 HIV-1 BaL, as described before (Moncunill et al., 2005). P24 antigen production was quantified by using an ELISA assay as described above.

2.7. 50% effective concentration (EC_{50}) and 50% cytotoxic concentration (CC_{50}) calculation

Effective concentrations that inhibit 50% of HIV infection and cytotoxic concentrations that induce 50% death of non-infected cells were calculated by a non-linear regression using the Enzfitter software.

3. Results

3.1. M-CSF stimulated αv-integrin expression in monocyte-derived macrophages

Freshly isolated monocytes did not express αv-integrins, but after 3 days of culture with M-CSF, monocytes acquired a differentiated phenotype characterized by an upregulation of αv-integrin expression. To characterize MDM receptor expression after 3 days of culture with M-CSF, CD14, CD4 and CCR5 expression was analyzed. More than 90% of the population expressed CD14 and CCR5 (96 ± 2% and 92 ± 2% respectively) and a mean of 80 ± 10% expressed CD4 (Fig. 1A). Integrin expression was analyzed in 3 day M-CSF differentiated MDM, using the specific anti-αv monoclonal antibody 17E6 (Fig. 1B). As shown in Fig. 1B, 57 ± 3% cells were detected positive. To confirm the αv-expression, we also analyzed MDM staining with the anti-αvβ6 mAb, 14D9.F8 (Mitjans et al., 1995), which showed 52 ± 3% of positive cells (Fig. 1C).

3.2. Anti-αv mAb 17E6 inhibited HIV-1 infection in acutely infected MDM

Freshly isolated monocytes could not be infected with HIV-1 (data not shown). Conversely, HIV p24 antigen production was dramatically increased after M-CSF stimulation. Thus, we studied the effect of 17E6 in HIV-1 BaL infection of MDM stimulated for 3 days with M-CSF. At day 7 post-infection, HIV-1 BaL production was analyzed. As expected, the gp41 inhibitor C-34 and the RT inhibitor AZT completely blocked p24 antigen production (Fig. 2A). The mAb 17E6 inhibited HIV antigen production by infected MDM. Inhibition was dose-dependent with a calculated 50% effective concentration (EC_{50}) of 17 ± 2 μg/ml (Fig. 2B). A mouse IgG1 antibody used as isotype control was not able to block HIV-1 BaL replication in acutely infected macrophages, suggesting that the anti-HIV activity shown by 17E6 was specific of its interaction with αv subunit (Fig. 2A). Cell viability was analyzed using the MTT-based colorimetric assay, a previously validated method at a concentration range from 100,000 to 1560 cells/well (data not shown). 17E6 was not toxic to MDM at the concentrations tested (Fig. 2B). Similar results were obtained at 14 days post-infection. At this time, the viability of HIV-infected cells was roughly 100% relative to uninfected control cells, confirming that MDM may survive and maintain a chronic virus infection (Aquaro et al., 2002).

3.3. Inhibition of HIV replication was dependent on the αv component of integrins

To evaluate the role of integrin components in HIV replication, 14D9.F8 (an anti-αvβ6 mAb) and 5C4 (an anti-β6 mAb) (Mitjans et al., 1995; Plow et al., 2000) were tested. The anti-αvβ6 mAb 14D9.F8, at 20 μg/ml, inhibited p24 antigen production by 76 ± 22% at day 7 without a detectable cytotoxic effect (Fig. 2A), whereas the anti-β6 mAb 5C4 did not show any significant anti-HIV activity at a concentration range from 0.1 to

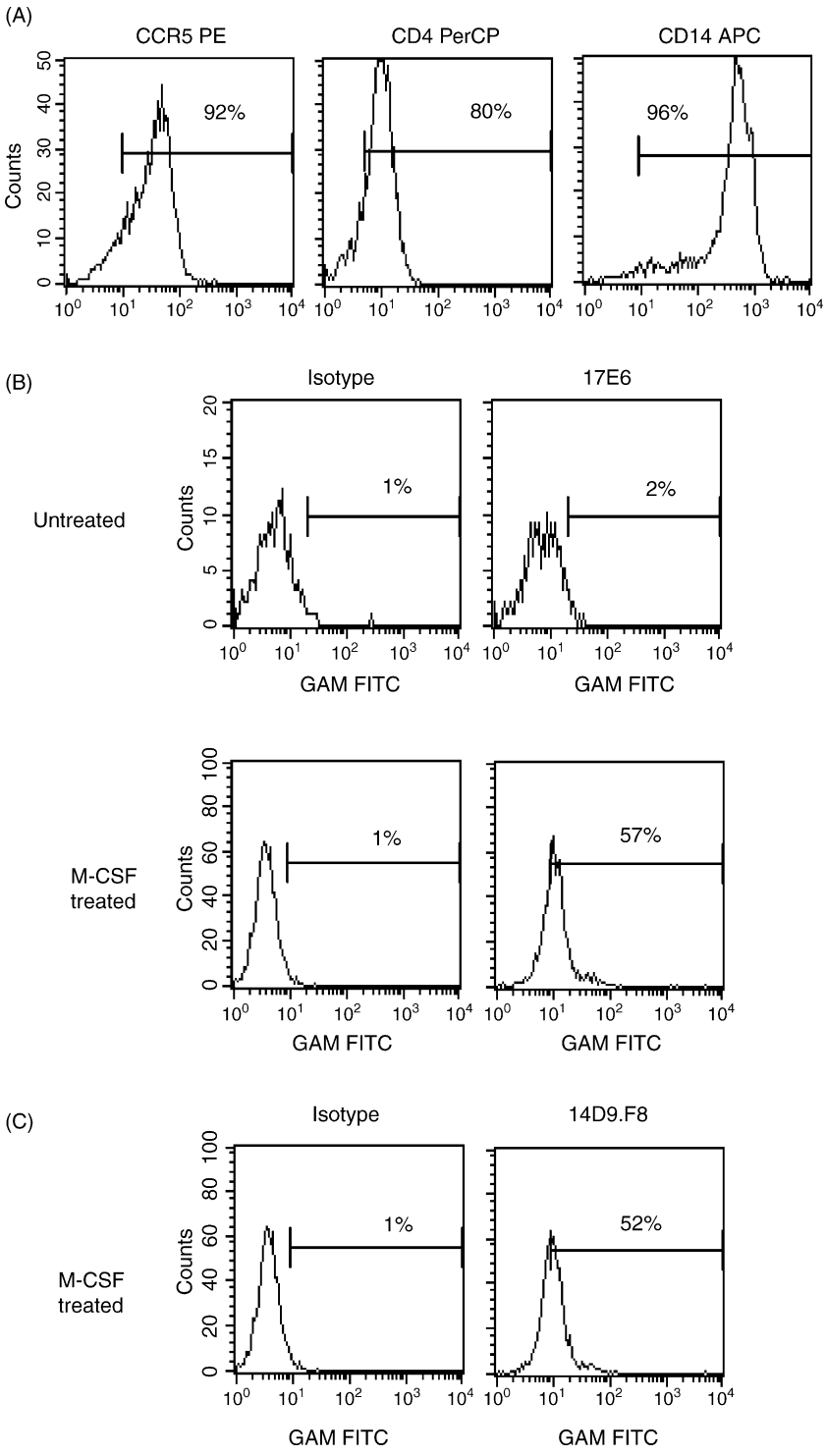

Fig. 1. αv-integrin expression in monocyte-derived macrophages. (A) Characterization of CCR5, CD4 and CD14 expression in M-CSF treated MDM. (B) 17E6 mAb staining of untreated or M-CSF treated MDM. (C) 14D9.F8 mAb staining to evaluate αvβ6-integrin expression in MDM treated with M-CSF. Values are the mean of three experiments.

40 μg/ml (data not shown). To discard any non-specific activity of the anti-αv mAb 17E6 and the anti-αvβ6 mAb 14D9.F8, we tested their antiviral activity in PBMC and lymphoid MT-4 cells, both negative for αv-containing integrin expression. Neither 17E6 nor 14D9.F8 showed anti-HIV activity in these cell lines at the highest concentration tested, 100 μg/ml [EC_{50} >100 μg/ml and 50% cytotoxic concentration (CC_{50}) >100 μg/ml]. Taken together, these results suggest that engagement of the αv component of integrins was a necessary element in the inhibitory effect of anti-integrin mAbs on HIV replication in MDM.

Next, we studied the antiviral activity of EMD 409849, a low-molecular-weight compound specifically directed against

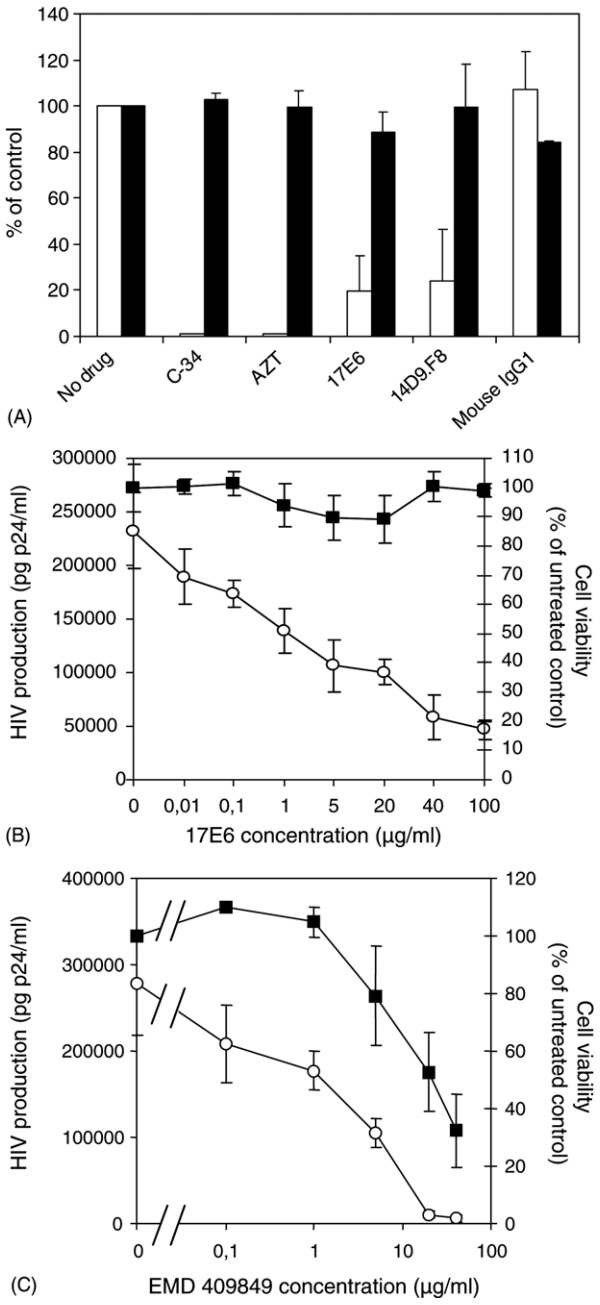

Fig. 2. Anti-HIV-1 activity of 17E6 in acutely infected monocyte-derived macrophages. MDM were infected with HIV-1 BaL in the presence of the indicated compounds and p24 antigen production was measured at day 7 post-infection. Simultaneously, cell viability was measured in drug or antibody treated but uninfected cells by the MTT method. (A) Anti-HIV-1 activity of C-34 (2 μg/ml), AZT (0.2 μg/ml), 17E6 (20 μg/ml), 14D9.F8 (20 μg/ml) and the isotype control mouse IgG1 (20 μg/ml). Empty bars represent p24 antigen production relative to infected control (No drug). Solid bars represent cell viability relative to the untreated control (No drug). Data represent the mean ± S.D. of five experiments. Each experiment was run in triplicate. (B) Antiviral activity (empty circles) and cell viability (solid squares) of (B) 17E6 or (C) EMD 409849 at different concentrations. Values are mean ± S.D. of triplicate samples. Figure shows one experiment representative of three.

αvβ6 integrin (Fig. 2C). EMD 409849 inhibited HIV-1 BaL replication with an EC_{50} calculated of 3 ± 1 μg/ml at day 7 post-infection. Nevertheless, cytotoxicity generated by this compound was not negligible with a CC_{50} of 19 ± 12 μg/ml, but at subtoxic concentrations, i.e. 1 μg/ml, EMD 409849 still inhibited HIV replication by 36 ± 5%. The calculated selectivity index (SI, CC_{50}/EC_{50}) of EMD 409849 was 6. EMD 409849 was also cytotoxic to PBMC and lymphoid MT-4 cells (both negative for αvβ6 integrin expression) with a calculated CC_{50} of 92 μg/ml and 68 μg/ml respectively; suggesting a non-specific cytotoxic effect that may be independent of αv-expression.

3.4. HIV-1 production was not inhibited by 17E6 in chronically infected MDM

Inhibition of HIV-1 BaL infection in acutely infected MDM led us to investigate if 17E6 could inhibit the release of p24 antigen in chronically infected MDM. We infected MDM cultured for 3 days with M-CSF. At day 15 post-infection, chronically infected cells were obtained and the anti-HIV activity of 17E6 was evaluated. C-34 and AZT, at the same concentration as in acutely infection experiments, did not inhibit p24 antigen production, confirming that new rounds of infection did not occur. Conversely, the HIV-1 protease inhibitor ritonavir (7 μg/ml) showed a clear inhibition (up to 85%) of p24 antigen production as shown before for protease inhibitors (Aquaro et al., 2004; Perno et al., 1998). 17E6 or 14D9.F8 (20 μg/ml) did not have an effect on virus production from chronically infected cells (Fig. 3).

3.5. 17E6 partially blocked HIV-1 entry in MDM

To explore the mechanism of action of 17E6 in early HIV infection of macrophages, the capacity of 17E6 to inhibit HIV-1 BaL entry into MDM was evaluated by quantitative real-time PCR. Proviral DNA production was measured by PCR amplification of the HIV-1 LTR region normalized to the PCR-amplified cellular RNAseP gene. 17E6 (40 μg/ml) and 14D9.F8

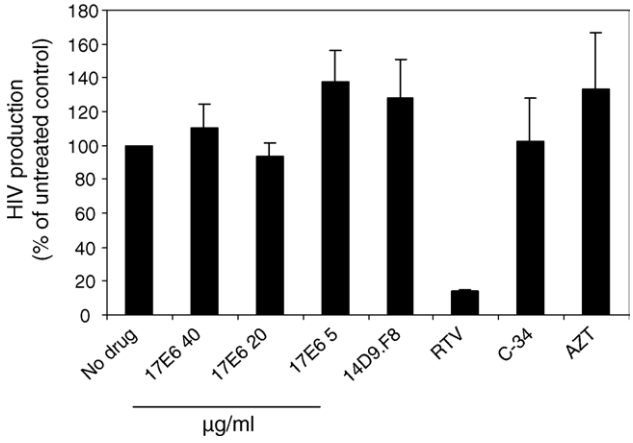

Fig. 3. 17E6 antiviral activity in chronically infected monocyte-derived macrophages. Chronically infected MDM were treated with the following drugs or antibodies: 17E6 at 40, 20 and 5 μg/ml, 14D9.F8 at 20 μg/ml, ritonavir (RTV) at 7 μg/ml, C-34 at 2 μg/ml and AZT at 0.2 μg/ml. Four days later, p24 antigen production in the culture supernatant was measured. Values are the mean ± S.D. of three experiments each run in duplicate.

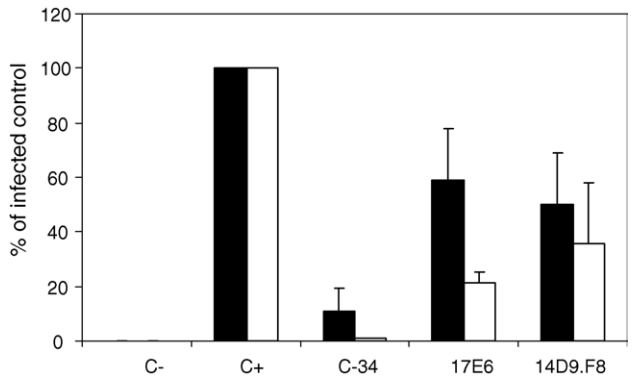

Fig. 4. HIV-1 entry in monocyte-derived macrophages. Inhibition of HIV-1 entry (solid bars) at 18 h after virus addition compared with p24-gag antigen production (empty bars) at day 7 post-infection. C-34 was used at 2 μg/ml, 17E6 at 40 μg/ml and 14D9.F8 at 20 μg/ml. Results are expressed relative to infected control (C+). Data are the mean ± S.D. of three experiments each one run in triplicate.

(20 μg/ml) blocked HIV-1 BaL entry in MDM by 41 ± 19% and 50 ± 19% of the infected control respectively (Fig. 4). Inhibition of proviral DNA detection was 89 ± 8% for C-34 (2 μg/ml). As expected, inhibition of proviral DNA was followed by inhibition of virus replication by 79 ± 4% for 17E6 at 40 μg/ml and 64 ± 22% for 14D9.F8 at 20 μg/ml (Fig. 4).

3.6. 17E6 downregulated receptor expression in MDM

Incubation of uninfected MDM with 17E6 induced a change in cell morphology in the absence of cytotoxicity (Fig. 5A). Untreated control cells showed an elongated fibroblastoid-like phenotype, characteristic of migratory cells, whereas 17E6 treated cells presented rounded morphology without cytotoxicity. Furthermore, 17E6, at 20 μg/ml, did not change the percentage of $CD4^+$, $CCR5^+$ and $CD14^+$ cells. However, the mean fluorescence intensity (MFI), that is cell surface density of these receptors, was decreased by 22 ± 4% for CD4, 36 ± 7% for CCR5 and 49 ± 6% for CD14 (Fig. 5B), as compared to untreated cells. Culture of MDM in the presence of 20 μg/ml of 17E6 did not induce the expression of CCL3, CCL4 or CCL5 (MIP-1α, MIP-1β and RANTES, respectively) as measured by cytokine bead array (BD Bioscience, Madrid, Spain) (data not shown). Taken together, these results suggest that the anti-HIV activity of 17E6 at an early stage of infection may be the consequence, at least in part, of a change in HIV receptor expression leading to reduced virus entry and replication.

4. Discussion

In the present study we have shown that engagement of αv-integrins reduced HIV infection in monocyte-derived

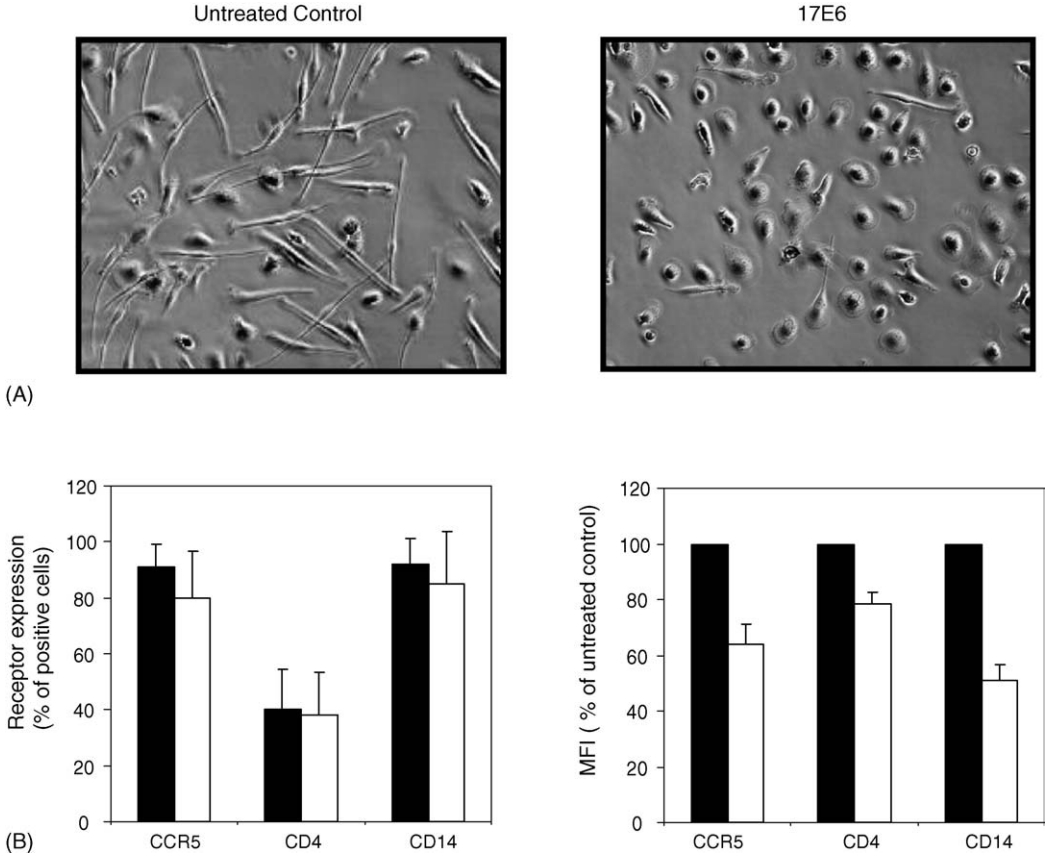

Fig. 5. The effect of 17E6 on cell morphology and HIV receptors. Monocyte-derived macrophages were cultured for 3 days with 17E6 at 20 μg/ml (empty bars) or without 17E6 (untreated control, solid bars). (A) Change of cell morphology after treatment with 17E6. (B) MDM were harvested and stained with the appropiate antibody and analyzed by flow cytometry. Percentage of positive cells for each receptor (left) and receptor cell surface density expressed in mean fluorescence intensity (MFI, right) were quantified. Data are the mean ± S.D. of three experiments.

macrophages. As previously described, the monocyte to macrophage differentiation process implies an upregulation of αv-integrins (De Nichilo and Burns, 1993). M-CSF is a factor that promotes the growth and the differentiation of immature cells of the macrophage lineage (De Nichilo and Burns, 1993) and enhances the capacity of HIV to infect and replicate in macrophages (Bergamini et al., 1994; Gruber et al., 1995; Kutza et al., 2000), probably through upregulation of CD4 and increased cell survival (De Nichilo and Burns, 1993). M-CSF induced the expression of αv-integrins (Fig. 1), a protein subfamily intimately involved in cell migration. A possible involvement of the αvβ3 integrin in the pathogenesis of HIV-1 in MDM has been previously reported (Lafrenie et al., 2002). Here, we provide further evidence that suggest a possible role of αv-chain of integrin receptors in the pathogenesis of HIV-1 in MDM. The specific anti-αv mAb, 17E6, was able to inhibit HIV-1 BaL replication in MDM in a dose-dependent manner (Fig. 2B), suggesting that the αv-chain may be a pharmacological target for HIV infection in macrophages. Furthermore, a mAb targeting αvβ6 integrin (14D9.F8) was also able to inhibit HIV-1 BaL replication (Fig. 2A). In contrast, a mAb targeting the β6 chain, 5C4, could not block p24 antigen production. These data would suggest that αvβ6 integrin and, specifically, its αv subunit may have an effect on the replication of HIV-1 in MDM. EMD 409849, a low-molecular-weight compound directed against αvβ6 integrin, partially inhibited HIV-1 replication (Fig. 2C). Chemical modification of this antagonist to circumvent its cytotoxicity could have a potential application.

To characterize the mechanism by which integrin function may contribute the infection of MDM by HIV, we analyzed the effect of 17E6 on HIV production by chronically infected MDM and on HIV entry in MDM. Our results suggest that αv-integrins may have an effect in the early stages of HIV-1 infection, as 17E6 did not inhibit p24 antigen release by chronically infected MDM (Fig. 3), but could reduce HIV-1 proviral DNA production (Fig. 4). 17E6 partially decreased the cell surface density of CD4 and CCR5 (Fig. 5B). It has been reported that CD4 density on cell surface could determine the susceptibility to HIV-1 infection in primary macrophages (Pesenti et al., 1999), and that cell surface CCR5 density could modulate the efficiency of post-entry steps, like retrotranscription, determining HIV-1 production in HOS cell line (Lin et al., 2002). These data would suggest that changes observed in CD4 and CCR5 receptor expression in MDM treated with 17E6 could modulate the efficiency of HIV infection. Nevertheless, further studies to evaluate the effect of the decrease of receptor expression on HIV replication would be needed to relate this result with the possible mechanism of HIV inhibition shown by 17E6. An alternative explanation for the mechanism of anti-HIV activity of integrin ligands has been proposed by Lafrenie et al., who suggested that HIV-gp120 in complex with CD4, could bind to cell surface αvβ3 integrin (Lafrenie et al., 2002).

Shi et al. (2004) have recently demonstrated that the integrin signaling pathway could be implicated in modulation of monocyte gene expression and its differentiation to macrophages. MAbs 17E6 (Fig. 5A) and 14D9.F8 (data not shown) change MDM morphology, suggesting the possibility that these agents were able to induce an intracellular signal, which, in turn, could be implicated in the inhibition of HIV replication. Consistent with this hypothesis, MDM treatment with 17E6 could downregulate cell surface density of CD14, CD4 and CCR5 (Fig. 5B). Furthermore, it has been described that αv-integrins, like αvβ3, are involved in phagocytic activity of macrophages (Stern et al., 1996). Engulfment of apoptotic bodies has been shown to enhance HIV-1 production in this cell type and the use of integrin RGD-antagonist can inhibit this enhancement (Lima et al., 2002). Therefore, it is also possible that 17E6 and 14D9.F8 could inhibit the phagocytic activity of MDM in cell culture with a concomitant decrease in cell infection and virus production.

Our results do not elucidate the mechanism by which αv-integrins modulate HIV-1 infection in MDM. Further characterization of the mechanism of action of integrin ligands, should help resolve their role in HIV infection. Of particular interest will be to decipher if other cytokines such as GM-CSF used to generate MDM (De Nichilo and Burns, 1993) or primary mature macrophages (e.g. alveolar macrophages) selectively express αv-integrins and become refractory to HIV infection by αv-integrin ligands. However, our results suggest an involvement of αv-integrins in the pathogenesis of HIV-1 in MDM. Modulation of the activity of these integrins may help to achieve new anti-HIV strategies to block HIV-1 infection in macrophages.

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