Integrin α5β1-Mediated Adenovirus Infection Is Enhanced by the Integrin-Activating Antibody TS2/16

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Adenovirus internalization generally has been accepted to involve an interaction of the adenoviral penton base protein with αvβ3 and αvβ5 cell surface integrins. In this study we show that exposure of a panel of melanoma cells to the β1-activating antibody TS2/16 rendered such cells more susceptible to adenovirus infection. This increase in adenoviral infectivity paralleled effects on cell adhesion, and both these characteristics were mediated, in part, by the α5β1 integrin. These observations suggest that α5β1 may act as an alternative adenovirus receptor and that integrin-activating strategies may improve the efficacy of recombinant adenoviruses as vectors for gene therapy.

The efficiency of adenovirus-mediated gene transfer depends on the biology of the recombinant virus-target cell interaction. The primary event in this sequence consists of virus-cell recognition and attachment, involving the fiber protein and host cell plasma membrane receptors (20). A secondary step of virus internalization depends on an interaction between the five conserved Arg-Gly-Asp (RGD) motifs in penton base protein (2, 3) and members of the integrin family of cell surface heterodimers (7, 9, 19, 20). At least eight of these different heterodimers have been shown to recognize RGD, including all five αv integrins and the integrins αIIbβ3, α3β1, and α5β1 (4, 10, 11, 18).

Despite the broad tissue tropism of recombinant adenoviruses it has become apparent that not all cells are equally susceptible to adenovirus infection. This variability has been thought to reflect the presence of specific integrins on the surface of target cells (7, 9, 19, 20). In the present study we have examined a range of human melanoma cell lines, known to vary in composition and level of cell surface expression of vitronectin receptors (12, 13), to determine whether this parameter affected sensitivity to adenovirus infection.

We exposed a panel of melanoma cell lines to hAd.CMV βgal, an E1-deleted, replication-deficient recombinant human adenovirus of serotype 5 (hAd5) which encodes the β-galactosidase gene under the transcriptional control of the cytomegalovirus (CMV) promoter at multiplicities of infection (MOI) of 1 to 50 (7). There was wide variation in recombinant adenovirus infection rates (Fig. 1) that was not accounted for by the relative expression of either αvβ3 or αvβ5. Thus, T8 cells, which were markedly less susceptible to adenovirus infection than either V(+)/B2 or DX3 cells, had large amounts of αvβ3 and αvβ5 at the cell surface (Fig. 1). Since VUP and V(+)/B2 cells express αvβ5, which acts as an alternative vitronectin receptor, we attempted to correlate adenovirus infection with total αv integrin expression. However, no such correlation was found, which would indicate that mechanisms unrelated to αvβ3 and αvβ5 integrin expression probably are involved in adenovirus infection of these cell types.

Simple cell surface expression of integrins is not necessarily predictive of their functional state. Thus, the integrins required for leukocyte adhesion to, and transmigration across, the endothelium during inflammation remain inactive until exposed to inflammatory mediators (17). Similarly, the platelet integrin αIIbβ3 is inactive on resting platelets but, during wound healing for example, is activated by thrombin or cytokines to initiate clot formation (15). We next sought to determine whether integrin activation status, as distinct from expression levels per se, had any effect on adenovirus infection. Experimentally, there are three principal methods for inducing activation of integrin-mediated adhesion: (i) exposure to the divalent cation Mn2+ (10); (ii) treatment with phorbol esters, including tetradecanoyl phorbol acetate (TPA) (6, 16, 21); and (iii) utilization of activating antibodies, such as the anti-β1 monoclonal antibodies TS2/16 (1, 14) and 8A2 (6). Figure 2 shows the results from a representative experiment where the melanoma cell lines HMB-2, VUP, and V(+)/B2 were exposed to increasing concentrations of MnCl2 in Tris-buffered saline during exposure to virus. Two of the three lines (HMB-2 and VUP) exhibited significantly increased virus infection in the presence of manganese compared to that of untreated cells (Student’s t test; P < 0.01 and P < 0.05 for VUP and HMB2, respectively). The V(+)/B2 line was unaffected by increasing Mn2+ concentrations.

Exposure of cells to phorbol ester (TPA; 100 to 400 nM for 30 min) also resulted in a significant increase in susceptibility to virus (data not shown). These treatments suggested that integrin activation status might be influencing virus infection. However, both manganese and TPA are relatively integrin nonspecific, since both treatments may affect a variety of cellular functions. The most direct way to evaluate the contribution of integrin function is to utilize an integrin-activating antibody. Accordingly we examined the effect of the β1-activating antibody TS2/16 (14), obtained from the American Type Culture Collection.

In order to confirm that antibody TS2/16 could enhance β1-integrin-dependent functions, adhesion assays were performed in the presence of this antibody. VUP, V(+)/B2, and HMB-2 cells were allowed to adhere to the β1-dependent...
FIG. 1. Adenovirus-mediated gene delivery to melanoma cell lines. Melanoma cell lines V(+)B2, VUP, HMB2, DX3, and T8 were seeded at 10^5 cells/well in 24-well plates and 24 h later were incubated with various amounts of Ad5.CMVβgal (MOI, 0 to 50) for 1 h at 37°C in serum-free Dulbecco's modified Eagle’s medium. After 48 h, the cells were stained with X-Gal (7) and the β-galactosidase activities of cells pretreated with TS2/16 were measured, and results were expressed as percent increase compared with untreated cells. Data are shown as means and standard errors of the means (n = 3), and one representative experiment out of four is shown. Ab, antibody.

FIG. 2. Effect of MnCl₂ on adenovirus-mediated gene delivery to HMB2, V(+)B2, and VUP cells. Melanoma cell lines were seeded at 10^5 cells/well in 24-well plates and 24 h later were exposed to MnCl₂ (0 to 5 mM in Tris-buffered saline, pH 7.4) during exposure to virus for 1 h at 37°C at an MOI adjusted to give a value of approximately 10% infection for each cell line when untreated with antibody. β-Galactosidase activities of cells pretreated with MnCl₂ were measured, and results were expressed as percent increase compared with untreated cells. Data are shown as means and standard errors of the means (n = 3), and one representative experiment out of four is shown.

FIG. 3. Effect of TS2/16 on adenovirus-mediated gene delivery to VUP, HMB2, and V(+)B2 cells. Melanoma cell lines were seeded at 10^5 cells/well in 24-well plates and 24 h later were pretreated with TS2/16 (American Type Culture Collection) at concentrations of 0 to 200 μg/ml in serum-free Dulbecco’s modified Eagle’s medium for 10 min at 4°C prior to exposure to virus for 1 h at 37°C at an MOI adjusted to give a value of approximately 10% infection for each cell line when untreated with antibody. β-Galactosidase activities of cells pretreated with TS2/16 were measured, and results were expressed as percent increase compared with untreated cells. Data are shown as means and standard errors of the means (n = 3), and one representative experiment out of four is shown. Ab, antibody.
infection of HMB-2 cells appears to be mediated by a fluorescent units were detected), and HMB2 (13.74 versus 14.02 fluorescent units were detected on untreated cells versus 14.67 g/ml had no effect on cell surface expression of α5 on VUP (14.1 fluorescent units were detected) cells as measured by flow cytometry, indicating that the effect of TS2/16 was not due to upregulation of α5β1.

In previous studies, adenovirus infection was reduced by preincubation of cells with fibronectin (20), indirectly suggesting that fibronectin receptors may mediate virus internalization. Our observation that α5β1, a major fibronectin receptor, participates in adenovirus infection provides a possible explanation for these earlier findings. It is possible that other fibronectin receptors may also participate in adenovirus infection. We found that V(+)B2 melanoma cells are four to five times more susceptible to adenovirus infection than are VUP melanoma cells. The cell line V(+)B2 is a high αvβ1-expressing derivative of VUP, expressing at least five times more αvβ1 than the parental cell line does (13), suggesting a possible role for αvβ1 in adenovirus internalization. However, the simultaneous administration of αv- and β1-blocking antibodies induced a similar level of inhibition of adenovirus infection to that obtained from the simultaneous administration of αvβ5- and α5-blocking antibodies. Therefore, if αvβ1 mediates adenovirus infection of V(+)B2 cells directly, its contribution is substantially smaller than the combined activity of α5β1 and αvβ5.

It is possible that αvβ1 has an indirect effect on α5β1, since cooperation between individual integrin heterodimers has been documented (4). Thus, we have shown previously that αvβ1 on V(+)B2 cells cooperates with α5β1 to mediate adhesion and spreading on fibronectin (13). The integrin αvβ3 also exhibits a similar behavior (15). Conceivably the increased susceptibility of V(+)B2 cells to adenovirus infection is due to the increased level of expression of αvβ1 enhancing the ability of the endogenous α5β1 to mediate adenovirus internalization.
In possible support of this, adenovirus infection of VUP cells was found to be αvβ5 dependent, but α5β1 independent (data not shown). Since VUP cells express levels of αvβ5 similar to those expressed by V(+)B2 cells (13), a possible explanation for this difference is the enhancing effect of αvβ1 in V(+)B2 cells on the activity of α5β1.

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