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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 αvβ1, 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 Mn^{2+} (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 $MnCl_2$ 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 Mn^{2+} 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

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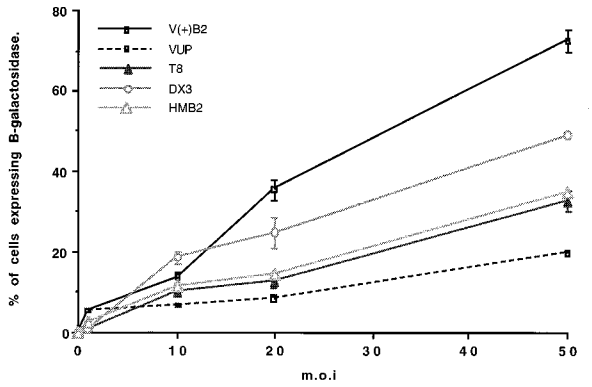

Median fluorescent units.

	αv	αvβ3	αvβ5	α5	β1
V(+)B2	61 +/-22	4 +/-6	11 +/-11	24 +/-5	184 +/-20
VUP	14 +/-6	2 +/-3	6 +/-6	17 +/-5	70 +/-8
T8	123 +/-8	30 +/-6	35 +/-11	53 +/-1	1144 +/-151
DX3	126 +/-26	38 +/-7	13 +/-2	113 +/-23	521 +/-139
HMB2	91 +/-22	62 +/-8	14 +/-9	13 +/-1	294 +/-35

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 percentage of β-galactosidase-positive (blue) cells were quantitated by light microscopy. A minimum of 500 cells were counted for each well. Data are shown as means and standard errors of the means (n = 3), and one representative experiment out of four is shown. The αv-integrin status of the human melanoma cell lines, determined by flow cytometry, is as described in the insert. Integrin levels are represented as median fluorescence units ± standard deviations.

substrates, fibronectin and collagen, in the presence of increasing concentrations of TS2/16. We found that both V(+)B2 and HMB-2 cells exhibited a TS2/16 dose-dependent increase in adhesion to the two substrates. Thus, at 200 μg of TS2/16 per ml, adhesion to fibronectin by V(+)B2 and HMB-2 cells was

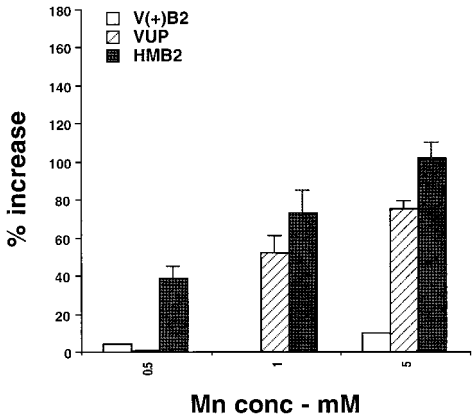

FIG. 2. Effect of $MnCl_2$ on adenovirus-mediated gene delivery to HMB2, V(+)B2, and VUP cells. Melanoma cell lines were seeded at 10^5 cells/well in 24-well plates and 24 h later were exposed to $MnCl_2$ (0 to 5 mM in Tris-buffered saline, pH 7.4) during exposure to virus for 1 h at 37°C at an MOI adjusted to give a value of approximately 10% infection for each cell line when untreated. β-Galactosidase activities of cells pretreated with $MnCl_2$ were measured, and results were expressed as percent increase compared with cells not treated with $MnCl_2$. Data are shown as means and standard errors of the means (n = 3), and one representative experiment out of four is shown.

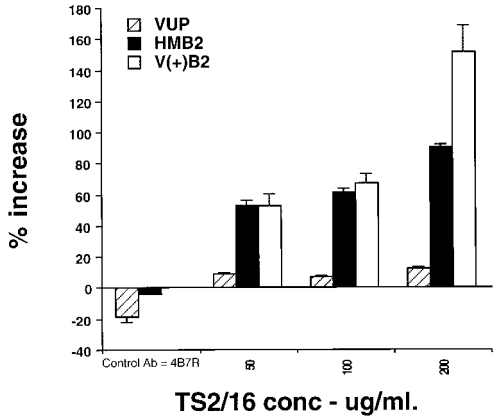

FIG. 3. Effect of TS2/16 on adenovirus-mediated gene delivery to VUP, HMB2, and V(+)B2 cells. Melanoma cell lines were seeded at 10^5 cells/well in 24-well plates and 24 h later were pretreated with TS2/16 (American Type Culture Collection) at concentrations of 0 to 200 μg/ml in serum-free Dulbecco's 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.

increased by 155 and 93%, respectively. In contrast, the presence of TS2/16 had no significant effect on the ability of VUP cells to adhere to either fibronectin or collagen.

We next investigated whether pretreatment of cells with TS2/16 enhanced the efficiency of adenovirus infection. The melanoma cell lines VUP, V(+)B2, and HMB-2 were incubated with increasing doses of TS2/16, or of a control non-activating antibody to β1, during a 60-min exposure to the hAd.CMV βgal virus. An antibody dose-dependent increase in the adenovirus-mediated gene transfer of β-galactosidase to V(+)B2 and HMB-2 cells was seen (Fig. 3). Thus, at 200 μg of TS2/16 per ml, the number of cells expressing β-galactosidase was increased by 150% in V(+)B2 cells and 98% in HMB-2 cells. VUP cell susceptibility to adenovirus infection was unaffected by the presence of up to 200 μg of TS2/16 per ml. Therefore, the TS2/16-dependent changes in susceptibility to adenovirus infection closely mirror, both in terms of magnitude and specificity, the TS2/16-dependent changes in β1-dependent adhesion reported above. These studies suggest that an increase in integrin ligand affinity was associated with enhanced susceptibility to adenovirus infection, probably by means of more efficient integrin-mediated virus internalization. This is supported by the observation that TS2/16 failed to increase either the virus infection or the cell adhesion of VUP cells. The inability of the control anti-β1 antibody to enhance adenovirus infection suggests that the ability of TS2/16 to increase virus infection was due to activation and not simply to aggregation of β1 integrins (Fig. 3).

The observation that adenovirus infection can be enhanced with an antibody to the β1 integrin subunit suggested that integrins other than the previously reported αvβ3 and αvβ5 play a role in adenovirus infection. Therefore, we examined the effects of a panel of integrin-blocking antibodies on the infection of both V(+)B2 and HMB-2 cells which had or had not been treated with TS2/16 at a final concentration of 100 μg/ml. Figure 4A indicates that, when used alone, the antibody most effective at inhibiting infection of untreated V(+)B2 cells was P1F6 (anti-αvβ5), which reduced infection by 57.5%. However,

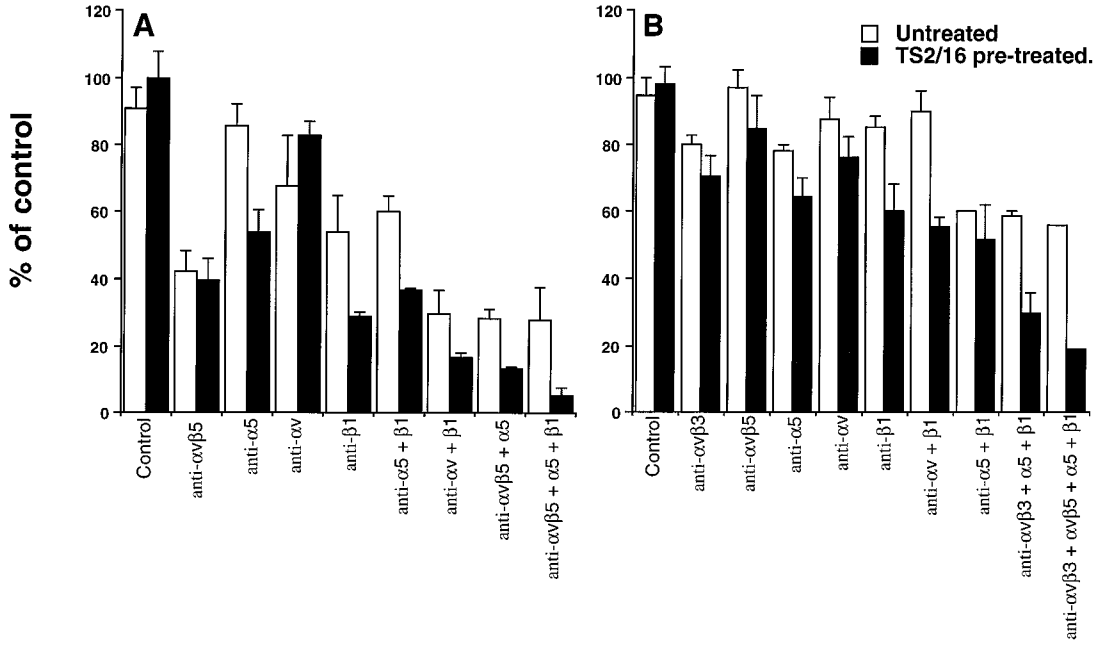

FIG. 4. Effect of function-blocking anti-integrin monoclonal antibodies on adenovirus-mediated gene delivery to V(+)B2 (A) and HMB2 (B) melanoma cell lines. Melanoma cell lines were seeded at 10^5 cells/well in 24-well plates, and 24 h later cells were left untreated (white bars) or were treated (black bars) with TS2/16 (100 μg/ml) in serum-free Dulbecco's modified Eagle's medium for 10 min at 4°C. Virus was introduced at an MOI adjusted to give a value of approximately 10% infection for each cell line when untreated with antibody. Cells were then treated for 10 min at 4°C with the following anti-integrin antibodies: anti-αvβ3 (LM609 [Chemicon]; dilution, 1:50), αvβ5 (P1F6), α5 (P1D6), β1 (P4C10 [Gibco Life Technologies]; dilution, 1:50), αv (L230 [American Type Culture Collection]; concentration, 10 μg/ml), and, as a control, 4B7R (5). The data are presented as percentages of the control cells (i.e., no antibody = 100%). Data for the control antibody are shown as the means and standard errors of the means for three individual experiments. All other data presented are means and standard errors of the means ($n = 3$) of one representative experiment.

inhibition of adenovirus infection was increased to 71.8% when P1F6 was combined with P1D6 (anti-α5), suggesting that α5β1 may be involved in adenovirus infection. This conclusion was supported by the response to integrin-blocking antibodies of TS2/16-activated V(+)B2 cells. Figure 4A shows that P1D6 alone had no effect on the adenovirus infection of untreated V(+)B2 cells, whereas P1D6 blocked adenovirus infection of TS2/16-activated V(+)B2 cells by 46%. Thus, treatment with TS2/16 enhanced the α5β1-dependent mechanism(s) for adenovirus infection. Adenovirus infection of TS2/16-treated V(+)B2 cells was abrogated almost completely (86.8% inhibition) by the coincubation of antibodies to αvβ5 and α5 during exposure to adenovirus. Thus, αvβ5 and α5β1 appear to be the major integrins involved in adenovirus infection of V(+)B2 cells.

The integrin α5β1 also mediates adenovirus infection of HMB-2. Figure 4B shows that significant inhibition of infection of HMB-2 cells (not treated with TS2/16) required combinations of integrin-blocking antibodies, thus blocking both α5- and β1-inhibited infection by 39.6%, and this level of inhibition was not increased significantly by also blocking αvβ3 and αvβ5. In contrast, adenovirus infection of TS2/16-treated HMB-2 cells was inhibited 48.3% by blocking both α5 and β1 and further inhibited by additionally blocking αvβ3 (70.3% inhibition) or αvβ3 plus αvβ5 (81.2% inhibition). Thus, adenovirus infection of HMB-2 cells appears to be mediated by α5β1, αvβ3, and αvβ5.

It should be noted that the presence of 100 μg of TS2/16 per ml had no effect on cell surface expression of α5 on VUP (14.1 fluorescent units were detected on untreated cells versus 14.67 on TS2/16-pretreated cells), V(+)B2 (18.05 versus 18.48 fluorescent units were detected), and HMB2 (13.74 versus 14.02 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 α5β1 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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