Regulation of epithelial and lymphocyte cell adhesion by adenosine deaminase–CD26 interaction

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The extra-enzymic function of cell-surface adenosine deaminase (ADA), an enzyme mainly localized in the cytosol but also found on the cell surface of monocytes, B cells and T cells, has lately been the subject of numerous studies. Cell-surface ADA is able to transduce co-stimulatory signals in T cells via its interaction with CD26, an integral membrane protein that acts as ADA-binding protein. The aim of the present study was to explore whether ADA–CD26 interaction plays a role in the adhesion of lymphocyte cells to human epithelial cells. To meet this aim, different lymphocyte cell lines (Jurkat and CEM T) expressing endogenous, or overexpressing human, CD26 protein were tested in adhesion assays to monolayers of colon adenocarcinoma human epithelial cells, Caco-2, which express high levels of cell-surface ADA. Interestingly, the adhesion of Jurkat and CEM T cells to a monolayer of Caco-2 cells was greatly dependent on CD26. An increase by 50 % in the cell-to-cell adhesion was found in cells containing higher levels of CD26. Incubation with an anti-CD26 antibody raised against the ADA-binding site or with

exogenous ADA resulted in a significant reduction (50–70 %) of T-cell adhesion to monolayers of epithelial cells. The role of ADA–CD26 interaction in the lymphocyte–epithelial cell adhesion appears to be mediated by CD26 molecules that are not interacting with endogenous ADA (ADA-free CD26), since SKW6.4 (B cells) that express more cell-surface ADA showed lower adhesion than T cells. Adhesion stimulated by CD26 and ADA is mediated by T cell lymphocyte function-associated antigen. A role for ADA–CD26 interaction in cell-to-cell adhesion was confirmed further in integrin activation assays. FACS analysis revealed a higher expression of activated integrins on T cell lines in the presence of increasing amounts of exogenous ADA. Taken together, these results suggest that the ADA–CD26 interaction on the cell surface has a role in lymphocyte–epithelial cell adhesion.

Key words: Caco-2 cells, cell-to-cell interaction, integrins, T lymphocytes.

INTRODUCTION

Adenosine deaminase (ADA), an enzyme involved in purine metabolism, is mainly a protein resident in the cytoplasm that also appears on the cell surface as an ecto-enzyme (ecto-ADA) [1]. In peripheral mononuclear blood cells, ecto-ADA is found on the majority of monocytes and B cells, and on 10–20 % of T cells [1]. The function of cell-surface ADA is to regulate the levels of extracellular adenosine or deoxyadenosine, which are toxic to lymphocytes [2]. In humans, the inheritable deficiency of ADA, caused by deletions, splicing defects or point mutations in the *ADA* gene, leads to severe combined immunodeficiency (SCID) [3–7]. Cell-surface ADA also has an extra-enzymic function. ADA via its interaction with cell-surface ADA binding protein CD26 can have a co-stimulatory effect in T-cell receptor (TCR)-mediated T-cell activation [8–12]. CD26 is a 105–110 kDa multifunctional cell-surface protein known to show an intrinsic dipeptidyl peptidase IV activity [13–16]. CD26 is constitutively expressed in a broad variety of cells, although the highest level of expression has been found in brush-border epithelia from the placenta, intestine and kidney [17,18]. In peripheral blood T cells, CD26 expression is highly regulated and enhanced upon T-cell activation [18–20].

On the basis of the existence of ADA–CD26 interaction, we postulated a role for cell-surface ADA in cell-to-cell adhesion. We have reported previously that ADA and CD26 co-localize on

the surface of T cells, although there are CD26 molecules that do not interact with endogenous ADA (ADA-free CD26) [8,10]. Using a heterologous system formed by human T lymphocytes (Jurkat and CEM T cells) and human epithelial cells (Caco-2 cells) as an adhesion model, we provide strong evidence that adhesion between T lymphocytes and Caco-2 cells is modulated by the interaction between ADA-free CD26 in lymphocytes and ADA expressed on the cell surface of epithelial cells.

The role of transductional events following the interaction of ADA and CD26 in cell-to-cell adhesion was also studied, since it is known that some adhesion receptors are activated via TCR/CD3 signalling and that ADA, via its interaction with CD26, has a co-stimulatory effect in TCR-mediated T-cell activation. The very late activation antigens (VLAs) or β 1 integrins function as cellular receptors for extracellular matrix proteins, and mediate intercellular adhesions [21–23]. These adhesion receptors can be regulated by different stimuli, such as extracellular bivalent cations, stimulatory monoclonal antibodies and cellular activation. Thus intracellular signals generated as a result of cell activation can regulate the affinity and conformation of integrins in many cellular systems [24,25]. We have subsequently examined, by incubation of T lymphocytes with Mn^{2+} (positive control) and exogenous ADA, whether CD26–ADA transduction signals can activate β 1 integrins. Our results shown that exogenous ADA is able to lead a significant increase in the level of activated integrins compared with control cells.

Abbreviations used: ADA, adenosine deaminase; SCID, severe combined immunodeficiency; TCR, T-cell receptor; VLA, very late antigen; A₁R, A₁ adenosine receptors; mAb, monoclonal antibody.

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Taken together, these results support the view that ADA-CD26 interactions may have an important role in cell-to-cell contacts that could be relevant in the development and function of lymphoid tissues.

MATERIALS AND METHODS

Materials

[³H]Thymidine (1 µCi/ml) was purchased from Amersham Life Science (Cleveland, OH, U.S.A.). Ecoscint H scintillation solution was from National Diagnostic (Atlanta, GA, U.S.A.). Calf ADA, which was filtered through a Sephadex G-25 column before all assays, was obtained from Boehringer Mannheim (Barcelona, Spain). Human plasma fibronectin was purchased from Sigma (St Louis, MO, U.S.A.). All other products were of the best grade available and purchased from Merck (Darmstadt, Germany) or Sigma Chemicals (St Louis, MO, U.S.A.).

Antibodies

Antisera raised against purified ADA and anti-peptide antisera against A₁ adenosine receptors (A₁R) were generated in our laboratory and characterized as described previously [40]. The antibody raised against A₁R (PC21) was purified from serum by affinity chromatography using the specific peptide coupled to Sepharose CL4B (Pharmacia Biotech AB, Uppsala, Sweden). Anti-ADA antibody was purified using ADA coupled to cyanogen-bromide-activated Sepharose (Pharmacia Biotech AB, Uppsala, Sweden). The anti-(human CD26) monoclonal antibody (mAb) Ta5.9-CC1-4C8 against the ADA-binding site in CD26 was generously given by Dr E. Bossman (Eurogenetics, Brussels, Belgium). The anti-(human dipeptidyl peptidase IV/CD26) mAb, 4H12, was obtained from Endogen Inc. (Boston, MA, U.S.A.). Anti-(LFA1-β) mAbs (anti-CD18 and LIA3/2), anti-(LFA1-α) mAbs (TP1/32 and TP1/40), anti-β₁ mAb Lia1/2 (CD29) and the mAb raised against activated integrin HUTS-21 were provided by Dr F. Sánchez (Hospital de la Princesa-U.A.M., Madrid, Spain). Sheep anti-(rabbit IgG) and FITC-conjugated goat anti-(mouse IgG) were from Sigma.

Cell lines

The human colon adenocarcinoma Caco-2 cell line (A.T.T.C., Rockville, MD, U.S.A.) was maintained in Dulbecco's modified Eagle's medium (Gibco, Life Technologies SA, Barcelona, Spain) supplemented with 10% (v/v) fetal bovine serum (Gibco), 1% (v/v) minimal essential medium (containing non-essential amino acids), 1 mM sodium pyruvate, 2 mM L-glutamine and antibiotics (100 units/ml penicillin and 100 µg/ml streptomycin).

Jurkat CD4⁺ cell clone J32 parental line, Jurkat cell clone #11, which overexpresses CD26 [8,32], CEM cell clones (CEM^{NO1}, CEM^{LO2} and CEM^{5C2}; obtained from Dr A. G. Hovanessian, Pasteur Institut, Paris, France, and characterized as described by Callebaut et al., [33]), and SKW6.4 (provided by Dr J. M. Moyano, Laboratori d'Investigació Merck Química, Barcelona, Spain) were grown in RPMI 1640 medium supplemented with 10% (v/v) fetal bovine serum, 2 mM glutamine and antibiotics (100 units/ml penicillin and 100 µg/ml streptomycin). Transfected cells (CEM and Jurkat #11 cells) were cultured in the presence of 0.5 mg/ml geneticin G-418 (Gibco).

Immunocolocalization by confocal microscopy and flow cytometry

Expression of cell markers was performed by immunofluorescence staining, as described elsewhere [34]. Subconfluent cells

[fixed with 4% (w/v) paraformaldehyde for 15 min] were incubated with fluorescein-conjugated anti-A₁R antibody (PC21-FITC, 50 µg/ml) and/or fluorochrome-conjugated anti-ADA antibody [anti-(ADA-TRITC) or anti-(ADA-FITC), 50 µg/ml] or rhodamine-conjugated anti-CD26 antibody (Ta1-RD1 1/10; Coulter Immunology, Hialeah, FL, U.S.A.) for 1 h at 37 °C. Samples were analysed in a FACScan flow cytometer (Becton Dickinson, Mountain View, CA, U.S.A.) or were mounted using immunofluorescence medium (ICN Biomedical Inc., Costa Mesa, CA, U.S.A.) and analysed by confocal microscopy (Leica TCS 4D confocal scanning laser microscope adapted to an inverted Leitz DMIRBE; Leica Lasertechnik GmbH, Heidelberg, Germany).

Integrin expression assays

Cells (10⁶ per ml) were washed twice in Hepes/NaCl buffer [20 mM Hepes/150 mM NaCl/2 mg/ml D-glucose (pH 7.4)] and were incubated in each Eppendorf with 10 µg/ml or 100 µg/ml ADA for 30 min at 37 °C in 500 µl of Hepes/NaCl buffer. After this treatment, cells were incubated for 20 min at 37 °C with the corresponding mAb (1:2 dilution of LIA or HUTS-21) in 50 µl of Hepes/NaCl buffer or Hepes/NaCl in the presence or absence of 1 mM MnCl₂. Cells were washed three times with 200 µl of Hepes/NaCl buffer and incubated for an additional period of 30 min at 4 °C with 75 µl of a 1:300 dilution of FITC-conjugated goat anti-(mouse IgG) secondary antibody (Sigma) in Hepes/NaCl buffer. After three washes with PBS, cells were fixed in 400 µl of 4% (w/v) paraformaldehyde in PBS and analysed with an EPICS Profile flow cytometer (Coulter).

Adhesion assays

Cell-to-cell adhesion assays were performed as described previously [23]. Briefly, Caco-2 cells were seeded at 1.5 × 10⁵ cells/well in six-well plates, and grown to confluence in Dulbecco's modified Eagle's medium. Cultured lymphocytes (SKW6.4 B cells, Jurkat T cells, Jurkat #11 T cells or CEM cell clones) were radiolabelled overnight with [³H]thymidine (0.2 µCi/ml), washed three times with PBS, and suspended at 10⁶ cells/ml in RPMI 1640 medium containing 0.6% (w/v) BSA and 10% (v/v) fetal bovine serum. Labelled cells (10⁶ cells/ml), pre-incubated for 30 min at 37 °C with medium alone (control) or with medium supplemented with anti-CD26 (Ta1-RD, 5 µg/ml or Ta5.9, 5 µg/ml), an anti-(LFA-1) antibody (1:100 dilution of anti-CD18, TP1/40 or TP1/32, or a 1:2 dilution of LIA3/2), an irrelevant antibody [goat anti-(rabbit IgG); 5 µg/ml] or ADA (10 µg/ml, 25 µg/ml or 50 µg/ml) were added to the confluent monolayers. After 1 h at 37 °C, non-adherent lymphocytes were harvested by gentle pipetting followed by two additional gentle washes with PBS. Remaining cells (epithelial cells plus adherent lymphocytes) were treated for 90 min with 1 ml of a 0.2% solution of SDS and transferred to scintillation vials containing 10 ml of scintillation fluid (Formula-989; NEN Research Products, Boston, MA, U.S.A.). Radioactivity was quantified in a β-counter (1600 TRI-CARB; Packard, Meriden, CT, U.S.A.) with 50% efficiency. The number of T-cells corresponding to the released radioactivity was determined from a linear relationship using radiolabelled T-cells lysed and processed as described above. Results were expressed as the percentage of adhesion compared with basal values. Each assay point was performed in triplicate.

Cell adhesion to fibronectin plates was quantified as previously described [26]. Briefly, 96-well flat-bottomed plates (Cultek, Madrid, Spain) were coated overnight at 4 °C with 10 µg/ml fibronectin. After saturation of wells with 1.5% (w/v) BSA in

PBS for 1 h at 37 °C, 1×10^5 Jurkat or Jurkat #11 cells were added to each well in the absence or presence of different concentrations of reagent. Plates were then transferred to a CO₂ incubator and incubated for 1 h at 37 °C. The percentage of cells that remained adhered after gentle washes of wells with PBS was calculated by measuring the absorbance of wells at 540 nm after fixation with 4% (w/v) paraformaldehyde and staining with 0.1% Crystal Violet.

RESULTS

Expression of ADA, CD26 and A₁R in Caco-2 cells and in lymphocyte cells

The distribution of ADA, CD26 and A₁R was analysed by confocal microscopy. As shown in Figure 1(A), the confocal images revealed a good CD26-ADA co-localization, although some cells appear to be only positive for cell-surface ADA (green cells). The bright ADA signal also co-localizes with the A₁R stain (Figure 1B). This result correlates well with the role of A₁R as an alternative ADA-binding protein in non-lymphoid cells [34–36].

Expression of cell-surface ADA and CD26 and the lack of expression of A₁R in Jurkat cells have been previously described [8,10,32]. Moreover, we have reported that ADA is present on the surface of different lymphocyte cell lines. Thus the highest percentage of expression was found in the SKW6.4-B-derived cell line, with the lowest occurring in Jurkat T-lymphoma-derived cells [8,10]. The expression of CD26 was analysed by flow cytometry in different CEM or Jurkat cell clones with different degrees of overexpression of CD26 (see the Materials and methods section). Parental cells (clone CEM^{NO1} and Jurkat) showed lower expression than the clone CEM^{LO2} or Jurkat #11 which overexpressed CD26 (Figures 1C and 1D). The means for the intensity of fluorescence of CD26 in parental Jurkat or CEM^{NO1} cells were 20 and 19 respectively, whereas the values for CD26-overexpressing Jurkat (Jurkat #11) or CEM (CEM^{LO2}) were 55 and 52 respectively. The expression values of ADA

(in terms of mean fluorescence intensity) for CEM^{NO1}, CEM^{LO2}, Jurkat and Jurkat #11 cells were 0.5, 3.5, 1 and 6.5 respectively. Individual differences between cells within a given cell line were found (see Figures 1C and 1D for expression of CD26 in Jurkat and CEM clones)

Cell-surface ADA on Caco-2 cells interacts with CD26 expressed in T lymphocytes

In order to study whether the ADA–CD26 module has a role in epithelial–lymphocyte cell adhesion, we performed a standard adhesion assay. Thus two different lymphocyte cell lines, Jurkat (a model of T lymphocyte) and SKW6.4 (a model of B lymphocyte), were adhered to epithelial monolayers. [³H]Thymidine-labelled Jurkat or SKW6.4 cells were incubated with confluent monolayers of Caco-2 cells for 45 min at 37 °C, as described in the Materials and methods section. Adhesion, expressed as adherent cells/cm² (mean for triplicates $\pm$ S.D. from five independent experiments), of Jurkat cells to epithelial Caco-2 monolayers was 7500 ± 400 . This value is markedly (over 2-fold) higher than that corresponding to adhesion of SKW6.4 cells (3100 ± 90). Jurkat cells and SKW6.4 cells display different amounts of ADA on their surface. Whereas SKW6.4 cells show a high expression level of ADA, the expression of CD26 in Jurkat cells is higher than cell-surface ADA (see above). Thus, on the Jurkat cell surface, there are a marked proportion of CD26 molecules that are not interacting with endogenous ADA (ADA-free CD26) [8]. Taking into account all these data, we hypothesized that adhesion between T cells and epithelial cells would be in part mediated by ADA-free CD26 on Jurkat cells and ADA expressed on Caco-2 cells. To address this hypothesis, we studied the effect of exogenous ADA upon adhesion of T lymphocytes to epithelial cells. Caco-2 cell monolayers were pre-incubated for 30 min in medium without or with ADA (10 μ g/ml), then [³H]thymidine-labelled Jurkat cells were added and incubated for 45 min at 37 °C, as described in the Materials and methods section. Adhesion of Jurkat cells, expressed as adherent cells/cm²

Figure 1 Expression of ADA, CD26 and A₁ adenosine receptor on the surface of the different cell lines

Cells were fixed [4% (w/v) paraformaldehyde] for 15 min and washed in PBS/20 mM glycine. Double immunofluorescence staining (A and B) or immunostaining with the fluorescein-conjugated anti-CD26 antibody (C and D) was performed as indicated in the Materials and methods section. (A) Surface expression of ADA (green) and CD26 (red) in Caco-2 cells. The CD26-ADA co-localization is indicated in yellow. (B) Surface expression of A₁R (green) and ADA (red) in Caco-2 cells. The A₁R-ADA co-localization is indicated in yellow. (C) Flow-cytometric analysis of CD26 in CEM^{NO1} (violet) and CEM^{LO2} (blue). (D) Flow-cytometric analysis of CD26 in Jurkat (maroon) and Jurkat #11 (blue). The red trace represents unspecific labelling.

Figure 2 Effect of exogenous ADA and anti-CD26 antibodies on the adhesion of Jurkat cells to epithelial monolayers

[³H]Thymidine-labelled Jurkat cells were pre-incubated with two different anti-CD26 antibodies (5 µg/ml 4H12 or 5 µg/ml Ta5.9), or with different concentrations of exogenous ADA (10 µg/ml or 25 µg/ml) or an irrelevant antibody (5 µg/ml) for 30 min at 37 °C. Adhesion assays were performed for 45 min at 37 °C, as described in the Materials and methods section. Percentages of adherent cells represent means for triplicate $\pm$ S.D. from five independent experiments (* $P \leq 0.01$).

Figure 4 Adhesion of cells overexpressing CD26 to epithelial monolayers

[³H]Thymidine-labelled lymphocytes (Jurkat, Jurkat #11, CEM^{N01}, CEM^{L02} and CEM^{L02}) were allowed to adhere to Caco-2 monolayers for 45 min at 37 °C, as described in the Materials and methods section. The basal adhesion in Jurkat and CEM^{N01} cells has been considered to be equivalent to 100% adhesion. Percentages of adherent cells represent means for triplicates $\pm$ S.D. from five independent experiments (* $P \leq 0.05$; ** $P \leq 0.001$).

Figure 3 Effect of anti-LFA1 antibodies on the adhesion of Jurkat cells to epithelial monolayers

(A) [³H]Thymidine-labelled Jurkat cells were pre-incubated for 30 min at 37 °C without (control) or with anti-(LFA1-β) antibodies (1:100 dilution of anti-CD18 or 1:2 dilution of LIA3/2) or anti-(LFA1-α) antibodies (1:100 dilution of TP1/32 or TP1/40). Cells were allowed to adhere to monolayers of Caco-2, as described in the Materials and methods section. In (B) Jurkat cells were pre-incubated (37 °C, 30 min) with anti-CD18 antibody (1:100 dilution), with ADA or with both reagents. Percentages of adherent cells relative to the control represent means for triplicates $\pm$ S.D. from five independent experiments (* $P \leq 0.01$).

(means for triplicates $\pm$ S.D. from five independent experiments), to non-treated monolayers was 7900 ± 300 , and a small but significant increase in the adherence of Jurkat cells to treated Caco-2 cells was observed (9900 ± 300). A similar experiment was performed by pre-incubating Jurkat cells with ADA (10 or 25 µg/ml) before addition of T cells on to the epithelial cell layer. CD26 expression on the Jurkat cell surface is not modified by pre-incubation with exogenous ADA (results not shown). Under these conditions, ADA decreased the T-cell adhesion to Caco-2 cells by $40 \pm 5\%$. A similar decrease ($50 \pm 5\%$) was obtained by pre-incubating Jurkat cells with a monoclonal antibody, Ta5.9, directed against the ADA-binding site in CD26 (Figure 2). An irrelevant antibody or an anti-CD26 antibody not directed against the ADA-binding site (4H12) did not modify the Jurkat–Caco-2 adhesion. These results suggest that cell-surface ADA from epithelial cells, via binding to CD26 on T cells, regulates cell-to-cell adhesion. The major known mechanism of T-cell adhesion is mediated by T cell integrin LFA-1. When Jurkat cells are incubated with antibodies raised against LFA-1, the adhesion is markedly reduced. This happens irrespective of whether the antibody is directed against LFA1-α or LFA1-β (Figure 3A). Interestingly, the effects of antibodies or of exogenous ADA were neither additive nor synergistic. In fact, pre-incubation of Jurkat cells with anti-CD18 antibody plus ADA led to a reduction in the adhesion similar to that obtained with any of the reagents alone (Figure 3B).

In order to verify that T-lymphocyte adhesion to epithelial cells depends upon the degree of expression of CD26 on lymphocytes, we compared the adhesion of parental Jurkat cells to Caco-2 cells with the adhesion of Jurkat cells overexpressing human CD26 (clone #11; see the Materials and methods section). Jurkat cells overexpressing CD26 increased their adhesion to Caco-2 cells by 50% compared with parental Jurkat cells. This result clearly indicates that the expression level of CD26 in Jurkat cells correlates with adhesion to epithelial cells (Figure 4). The same experiment was performed with CEM T cells. We found a similar adhesion pattern, with the highest level of

Figure 5 Effect of ADA and an anti-CD26 antibody on the adhesion of Jurkat and Jurkat #11 cells to epithelial monolayers

[³H]thymidine-labelled lymphocytes were pre-incubated (for 30 min at 37 °C) with ADA (10 µg/ml, 25 µg/ml or 50 µg/ml) or Ta5.9 (5 µg/ml) an anti-CD26 mAb directed against the ADA-binding site. Cells were allowed to adhere to Caco-2 cells as described in the Materials and methods section. Percentages of adherent cells represent the means for triplicates $\pm$ S.D. from five independent experiments (* $P \leq 0.01$; ** $P \leq 0.001$).

Figure 6 Expression of the β_1 integrin on Jurkat cells induced by ADA incubation

Jurkat #11 cells pretreated with Hepes buffer in the absence (control) or presence of Mn^{2+} (1 mM) or ADA (100 µg/ml) were stained with the HUTS-21 antibody [31] and analysed by flow cytometry, as described in the Materials and methods section. Left to right: control, ADA, Mn^{2+} -treated cells profiles are shown.

adhesion in CEM^{LO2} cells (which display high CD26 levels), an intermediate level in CEM^{NO1} (which has a basal CD26 expression) and the lowest in clone CEM^{5C2} (which is transfected with CD26 antisense cDNA) (Figure 4). As in the case of parental Jurkat cells, adhesion of Jurkat #11 diminished upon pre-incubation with the anti-CD26 mAb Ta5.9 directed against the ADA-binding site. Ta5.9 reduced by 52 % the adhesion of parental Jurkat cells to Caco-2 cells, whereas the reduction was higher (83 %) in the case of Jurkat #11 cells (Figure 5). These data reveal again that the participation of the ADA-CD26 module upon the overall adhesion increased in parallel with the expression of ADA-free CD26 in T cells. This was also confirmed by the drastic reduction in binding of Jurkat #11 cells to Caco-2 cells when the Jurkat cells overexpressing CD26 were incubated with increasing amounts of exogenous ADA (Figure 5). The maximal ADA-induced reduction in adhesion of parental Jurkat cells to Caco-2 cells was about 50 % compared with untreated cells (Figure 5).

ADA induces integrin activation in Jurkat cells

To characterize the mechanism underlying the CD26-ADA modulated cell-to-cell adhesion, we examined integrin activation in Jurkat cells. VLA integrins function as cellular receptors for extracellular matrix proteins, and mediate intercellular adhesions [21–23]. It has been reported that expression of the HUTS-21 epitope correlates with functional activation of VLA integrins, and that Mn^{2+} induces its exposure [26–31]. We studied whether the ADA-CD26 interaction can induce VLA activation similarly to well-characterized stimuli, such as bivalent cations. T parental cell lines (Jurkat and CEM^{NO1}) and clones overexpressing CD26 (Jurkat #11 and CEM^{LO2}) were pre-incubated with Mn^{2+} (positive control), with either exogenous ADA or Hepes buffer for 30 min at 37 °C. Activated integrins were detected using the mAb HUTS-21 and flow-cytometry analysis. Treatment with Mn^{2+} resulted in a marked increase in the expression of the HUTS-21 epitope on all cell lines assayed. When cells were pre-incubated with ADA, there was also a marked increase in the percentage of cells expressing integrins in an active conformation (Figure 6). In parental cells, the maximum effect (70–75 % of cells expressing the HUTS-21 epitope) was already achieved with 10 µg/ml ADA, while higher concentrations of ADA were necessary to obtain substantial integrin activation on CD26-overexpressing cell clones (Table 1). None of the stimuli caused major changes in the total expression level of β_1 as detected using the mAb LIA, indicating that Mn^{2+} - and ADA-induced effects were related to structural conformational changes, rather than to protein expression levels (Table 1).

Table 1 Integrin activation by ADA/CD26 interaction

The results are expressed as the percentage of cells expressing the HUTS-21 epitope. Values represent the means $\pm$ S.E.M. from four separate experiments performed in duplicate. The percentage of cells expressing β_1 integrin (activated or not) was assayed using the LIA mAb.

Stimuli	Cell type ...	% Cells labelled with HUTS-21				% Cells labelled with LIA			
		Jurkat	Jurkat #11	CEM ^{NO1}	CEM ^{LO2}	Jurkat	Jurkat #11	CEM ^{NO1}	CEM ^{LO2}
Hepes		42 $\pm$ 3	28 $\pm$ 3	41 $\pm$ 3	41 $\pm$ 3	95 $\pm$ 8	94 $\pm$ 8	95 $\pm$ 3	67 $\pm$ 3
ADA (10 µg/ml)		75 $\pm$ 3	30 $\pm$ 4	70 $\pm$ 3	49 $\pm$ 3	95 $\pm$ 10	93 $\pm$ 9	70 $\pm$ 3	67 $\pm$ 5
ADA (100 µg/ml)		—	60 $\pm$ 3	—	60 $\pm$ 3	—	98 $\pm$ 9	—	71 $\pm$ 3
Mn^{2+}		96 $\pm$ 7	81 $\pm$ 4	99 $\pm$ 7	99 $\pm$ 5	100 $\pm$ 10	90 $\pm$ 10	96 $\pm$ 3	66 $\pm$ 3

Figure 7 Adhesion of Jurkat cells to fibronectin

Cells (10^5) were incubated in the absence or presence of different concentrations of ADA in 96-well flat-bottomed fibronectin-coated plates (see the Materials and methods section). The number of Jurkat cells adhered to the plates is given as a percentage relative to the cells adhered in the absence of ADA (6000 ± 2000 cells). Values represent the means $\pm$ S.E.M. for two separate experiments performed in quintuplicate. * $P < 0.2$; ** $P < 0.05$.

To characterize further the role of ADA–CD26 interaction in integrin activation, we studied the effect of ADA in an *in vitro* model of adhesion to fibronectin-coated plates. Jurkat cells were incubated with exogenous ADA, and its adhesion to fibronectin-coated plates was analysed (Figure 7). The effect of ADA was dose-dependent, with an EC_{50} value of 10 ± 3 μ g/ml. Maximum cell adhesion to fibronectin plates was attained at a concentration of 13 μ g/ml.

DISCUSSION

The role of cell-surface ADA via its interaction with CD26 in T-cell activation has been well characterized [2,8,11,12]. In view of its localization on the cell surface, ADA would be a good candidate to participate in intercellular events, such cell-to-cell adhesion.

In this study we investigated the role of the ADA–CD26 module at the cell surface in human lymphocyte–epithelial cell adhesion. A good correlation between expression of ADA-free CD26 (CD26 molecules not interacting with endogenous ADA) and adhesion to Caco-2 cells was found. Thus SKW6.4 cells, model B cells that express 90–95% of ADA on their surface, adhere less than Jurkat cells, which have a pool of ADA-free CD26. Moreover, clones expressing high levels of CD26 (Jurkat #11 and CEM^{LO2}) show higher adhesion than the parental cell lines (Jurkat and CEM^{NO1}) or cells transfected with CD26 antisense cDNA (CEM^{5c2}). These results indicate that the contribution of the ADA–CD26 interaction to the overall adhesion of T cells to epithelial cells depends upon the degree of expression of ADA-free CD26 on the T cell surface. From a physiological point of view, it seems that events leading to the increase in CD26 expression, such as T-cell activation [8,10], would enhance the interaction between T cells and ecto-ADA-expressing cell types. The loss of adhesion when T cells were incubated with exogenous ADA, and the results obtained with Ta5.9, an antibody directed against the ADA-binding site in CD26, are also strongly indicative that blockade of the ADA-binding site in CD26 affects adherence between T cells and epithelial cells. It should be noted that the adhesion of Jurkat cells overexpressing CD26 is reduced by > 75% by pre-incubation with exogenous ADA or Ta5.9 antibody. When Jurkat

cells are incubated with antibodies directed against LFA1, the adhesion is markedly reduced. Owing to the fact that the effect of antibodies or exogenous ADA was neither additive nor synergistic, T cell LFA1 mediates the adhesion stimulated by CD26–ADA. Overall, these results support the view that the ADA–CD26 interaction has a relevant and specific role upon adhesion of human T lymphocytes to human epithelial cell layers, and is likely to be involved in the initial steps of adhesion of T cells to epithelial cells.

We have demonstrated that ADA on the surface of Caco-2 cells is anchored to either CD26 or A₁R. Although the stoichiometry of the ADA–CD26 interaction is not exactly known, it is assumed that CD26 displays a single ADA binding region, and that ADA is a monomer [37]. It is thus unlikely that ADA is bound to two CD26 molecules, i.e. one in each cell type. This may suggest that the ADA in Caco-2 cells that interacts with CD26 in lymphocytes is that which is bound to the A₁R. This would constitute a new role for A₁R as anchoring molecules able to present ADA to CD26 on the surface of contacting cells.

Adhesion between two different cell types is a complex phenomenon that requires a variety of extracellular matrix components and proteins on the surface of the interacting cells. ADA–CD26 recognition does not allow, in itself, efficient adhesion between lymphocytes and epithelial cells. In fact, the Jurkat cells bound to ADA-coated plates are not able to resist becoming dislodged during the washing steps required to remove unbound cells (results not shown). Thus a likely scenario in lymphocyte–epithelial cell interactions is the existence of early events mediated by the ADA–CD26 module, followed by activation of integrins, which are necessary for effective adhesion [38,39]. Interestingly, our data support the view that ADA–CD26 recognition leads to signalling pathways in lymphocytes which lead to integrin activation. In fact, CD26-mediated signalling via the ADA–CD26 interaction leads to a marked increase in the percentage of cells expressing the HUTS-21 epitope.

The role of the ADA–CD26 module in cell-to-cell recognition by lymphocytes is probably not restricted to the interaction with epithelial cells, but also involves other cell types expressing cell-surface ADA. There are a broad variety of non lymphoid cells, such as epithelial, muscular and renal cells, expressing ADA anchored to adenosine receptors. The ADA–CD26 module would be important for the interaction between these cells and lymphocytes. In such heterologous interactions, the ADA–CD26 module would be involved in the first steps of cell-to-cell recognition, and it would subsequently contribute, by signalling, to the engagement of the machinery required to switch integrins over to their active conformations in T cells. We are therefore seeking to examine whether the lack of cell-surface ADA on stromal cells could be the cause of defects in cell-to-cell recognition, which are necessary for proper immune organ development. This could be one of the mechanisms involved in the origin of severe combined immunodeficiency.

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