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LFA-1-mediated leukocyte adhesion regulated by interaction of CD43 with LFA-1 and CD147

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Abstract

The activity of the lymphocyte-function associated antigen 1 (LFA-1; CD11a/CD18) must be tightly controlled during the onset of cellular immunity. It is well known that the sialoglycoprotein CD43 can influence LFA-1 mediated cell adhesion in an either anti- or pro-adhesive manner through mechanisms not well understood. By using a yeast-2-hybrid screen and co-immunoprecipitation we identified physical association of CD43 with two novel partners, LFA-1 itself and the Ig-family member CD147 (EMMPRIN, basigin), and characterized how these interactions are involved in LFA-1-mediated cell adhesion. Monoclonal antibodies (mAbs) to both CD43 and CD147 induced similar homotypic cell aggregation and adhesion of Jurkat T cells and U937 myeloid cells. Both CD43 and CD147 mAbs induced dynamic co-capping of LFA-1 together with the CD43 and the CD147 molecule to cell contact zones. However, in contrast to CD43, we were not able to co-immunoprecipitate LFA-1 with CD147, which indicates that CD43 interacts with CD147 and LFA-1 in two distinct but similarly reorganized complexes. Co-transfection of CD43 interfered with the CD147-induced cell adhesion and aggregation, and siRNA-mediated knock down of CD43 in human T cells resulted in enhanced LFA-1 activation induced via CD147 and also the T cell antigen receptor. These results indicate that triggering CD43 and the underlying signaling pathways enhances LFA-1 adhesiveness while CD43 negatively regulates LFA-1 induction via other receptors by dynamic interaction with either LFA-1 or CD147.
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Keywords: LFA-1; CD147; CD43; Cell adhesion; Human leukocytes

1. Introduction

The lymphocyte-function associated antigen 1 (LFA-1; CD11a/CD18) is essential for the action of human leukocytes, including lymphocyte recirculation, migration into inflammatory sites and recognition of foreign antigens (Pribila et al., 2004). LFA-1 mediates a variety of homotypic and heterotypic cell adhesion events between leukocytes, antigen presenting cells (APCs), endothelial cells within blood vessels and other cells within tissues. The major ligands for LFA-1 are the intercellular adhesion molecules (ICAM)-1-3, with ICAM-1 (CD54) having the highest affinity (de Fougerolles et al., 1994). The LFA-1-mediated cellular contacts are highly dynamic and in most cases transient, therefore they are proposed to be a subject of tight regulation. Integrin avidity depends on modulation of integrin affinity and valency via "inside-out"-signals initiated by G protein-coupled receptor, cytokine and T cell receptor mediated signals (Dustin et al., 2004). While all of these signals can rapidly enhance LFA-1 avidity, there is also the need for coordinated de-adhesion of immune cells. Deactivation of LFA-1 and disassembly of LFA-1-mediated cell contacts seem to be vital for the generation of normal immune responses (Semmrich et al., 2005).

CD43, a major leukocyte cell surface sialoglycoprotein, is one of the potential mediators of LFA-1 de-adhesion. Its high level of glycosylation and net negative charge leads to an extended conformation of the extracellular domain enabling

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repulsion of cell contacts (Ostberg et al., 1998). Indeed, cells of CD43 knock out mice showed a substantial enhancement in both homotypic adhesion and ability to bind different ligands, including fibronectin and ICAM-1 (Manjunath et al., 1993; Manjunath et al., 1995). Furthermore, CD43 was shown to exert a negative regulatory effect on T cell trafficking by interfering with L-selectin-mediated adhesion (Stockton et al., 1998). Recently, it was shown that CD43 exerts its negative regulatory function on cell adhesion also in mast cells (Drew et al., 2005). However, the understanding of the physiological role of CD43 has been complicated by numerous reports showing rather pro-adhesive functions for CD43. Interestingly, CD43 itself was described to bind ICAM-1 (Rosenstein et al., 1991), which might be important for the initial interaction of T cells with other cells. We and others demonstrated the induction of cell aggregation upon treatment with CD43 monoclonal antibodies (mAbs) (Nong et al., 1989; Rosenkranz et al., 1993; Sanchez-Mateos et al., 1995). On the other hand, a number of CD43 mAbs could block cellular interactions, like T cell conjugate formation with certain cell types (Stöckl et al., 1996), or T cell binding to lymph node and Peyer's patch high endothelial venules (McEvoy et al., 1997). We hypothesized that the dynamic interactions of CD43 with additional membrane partner molecules could explain these contradictory and puzzling anti- and pro-adhesive properties of CD43 towards LFA-1. Indeed, there is evidence for several counter-receptors and ligands of CD43 including MHC class I (Stöckl et al., 1996) and sialoadhesin (van den Berg et al., 2001).

To find such potential regulators we performed a yeast-2-hybrid-screen (Y2H), co-immunoprecipitations and confocal microscopy. As a result of these experiments we can show here that CD43 interacts directly with LFA-1 as well as with CD147, a human Ig-family member (Kasinrerk et al., 1992). During the last couple of years, CD147 appeared to be a pleiotropic molecule involved in cell metabolism, cell adhesion and cell migration (Berditchevski et al., 1997; Cho et al., 2001; Kasinrerk et al., 1999; Kirk et al., 2000; Pushkarsky et al., 2001; Sun and Hemler, 2001; Wilson et al., 2002; Xu and Hemler, 2005; Yurchenko et al., 2001; Yurchenko et al., 2002). We show here that CD147 directly interacts with CD43 and that treatment of cells with both CD43 and CD147 mAbs induces a dynamic rearrangement of CD43, CD147 and LFA-1 to the areas of cell-cell contact. Moreover, the CD147-mediated adhesiveness is blunted by CD43 coexpression. Consistent with the latter finding, CD43 knock down by siRNA enhances CD147-mediated cell adhesion of human T cells to the LFA-1 ligand ICAM-1. Taken together, these data indicate that CD147 is an important co-receptor of CD43 in regulation of LFA-1-mediated cell adhesion.

2. Materials and Methods

2.1. Cells

The human monocytic cell line U937, the human T cell line Jurkat and the mouse thymoma cell line BW5147 were maintained in RPMI-1640 medium (Sigma, St. Louise, MO) supplemented with 100 μg/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine and 10% heat inactivated FCS (PAA, Linz, Austria). All cells were grown at 37 °C and 5% CO_2 in a humidified atmosphere. Cells were passaged three times a week.

2.2. Antibodies

The mAb used in this study were produced in our laboratories and described earlier with the exception of the IgG1 mAb MEM-257: The IgG1 mAb MEM-48 to CD18 in (Bazil et al., 1990), the IgG1 mAb MEM-59 to CD43 in (Stefanova et al., 1988), and the IgG1 mAbs MEM-M6/1, MEM-M6/3 and MEM-M6/8 to CD147 in (Koch et al., 1999). AFP-01 (IgG1) to human alpha-fetoprotein served as negative control.

2.3. Yeast-2-Hybrid screen

A human Jurkat T cell leukemia cDNA library cloned in the *Y2H* vector pB42AD (Clontech) was screened according to the manufacturer's instruction for CD43 interaction partners using the intracellular domain of CD43 (amino acids 277–400) as bait. Bait plasmids containing p53 and lamin were used as controls. Positive clones were selected upon Gal/Raf-induced expression of the cDNA library by using selective growth media. (SD/G/-WH = synthetic dropout agar medium with glucose, lacking tryptophan and histidin; SD/G/-WHUL = synthetic dropout agar medium with glucose, lacking tryptophan, histidin, uracil and leucin; SD/GR/-WHUL = synthetic dropout agar medium with galactose/raffinose, lacking tryptophan, histidin, uracil and leucin).

2.4. Immunoprecipitation

Cells (2×10^7 cells/ml) were lysed with lysis buffer (20 mM Tris-HCl, 150 mM NaCl, pH 7.5) containing 1% Brij58 as detergent and a set of protease inhibitors as described (Godar et al., 1999) for 30 min at 4 °C. Insoluble nuclei were removed by short centrifugation. Immunoprecipitation was performed on a 96 well plate (Maxisorb for ELISA; Nunc), which was coated before with 10 μg/ml goat anti-mouse Ig (H + L) (Caltag, Burlingame, California) in Tris-HCl buffer (pH 8.2) for 2 h at 37 °C. After washing three times with PBS, the plates were incubated with 20 μg/ml specific mAbs in PBS at 37 °C for 2 h. Afterwards, the plates were blocked with 2.5% BSA in PBS for 1 h at 37 °C. After the coating procedure, the cell lysates were incubated overnight at 4 °C. The plates were then washed twice with cold lysis buffer. Precipitated proteins were eluted by SDS-PAGE sample buffer and analyzed by SDS-PAGE and immunoblotting.

2.5. Cell adhesion assays

For homotypic cell aggregation, the cells were incubated for given time points in a 96-well plate (3×10^4 cells/well) in the presence or absence of 10 μg/ml indicated mAbs. The degree of cell aggregation was scored as previously described (Kasinrerk et al., 2000). Briefly, 0 = no aggregation (>90% of cells were not aggregated); 1+ = the majority of cells were not aggregated, but a few small clusters of <20 cells were observed (this level of adhesion is typical of the spontaneous adhesion exhibited

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by many lymphoblastoid cell lines, including Jurkat); 2+ = 50% of cells were in medium to large size aggregates (20–50 cells) with the remainder as single cells; 3+ = nearly all cells were aggregated in medium to large size aggregates (>50% cells) with only a few (<20%) non-aggregated cells; and 4+ = >90% of the cells were in large aggregates. Photographs were taken with a Nikon camera under an inverted microscope.

Cell adhesion to ICAM-1 was analyzed using an ICAM-1 receptor globulin (ICAM-1Rg) fusion protein (Kolanus et al., 1996), kindly provided by Dr. Waldemar Kolanus, University of Bonn, Germany, coated on 96 well plates. Briefly, donkey-anti-human IgG (Jackson Immunoresearch, West Baltimore Pike West Grove, USA) in PBS (pH 8.7) was coated on the plates at a final concentration of 10 μg/ml for 2 h at 37 °C. After washing, the plates were incubated with cell supernatant containing ICAM-1Rg fusion protein. Then, the plates were blocked with 1% BSA in PBS. The cells were added to the plates at a concentration of 1×10^5 cells/well in RPMI-1640 medium supplemented with 1 mM $CaCl_2$, 2 mM $MgCl_2$, 0.5 mM $MnCl_2$, 20 mM HEPES and 10 μg/ml indicated mAbs. Cells were incubated on the plates for 1 h at 37 °C. Unbound cells were washed away using PBS. The remaining cells were fixed on the plate by methanol for 15 min and then stained with 0.1% crystal violet. After lysing the cells with 0.5% Triton-X 100 the number of adherent cells was evaluated as a function of crystal violet absorbance at 595 nm by an ELISA reader.

2.6. siRNA transfection

Jurkat cells (5×10^6) were transfected with 1.5 μg CD43 siRNA (5′- GGAAGUUUCCAUCAAGAUG –3′, Ambion Diagnostic, Austin, TX USA, #16704) or scrambled siRNA (Ambion, #4611) in 100 μl cell line nucleofector kitV solution (Amaxa, Cologne, Germany). The transfection program C16 was used for electroporation. Transfected cells were grown in normal growth medium and transfected a second time after 4 days in culture. For functional assays, the silenced cells were used within 7 days after the first transfection. We had to perform two rounds of siRNA transfection in order to get a significant knock down efficiency, probably due to the slow protein turnover rate of CD43. One week after the first transfection, this procedure resulted in 75% reduced CD43 surface staining compared to cells transfected with control siRNA (Fig. 6A).

2.7. Confocal microscopy

Cover slips were coated with 20 μg/ml of CD147 mAb MEM-M6/8 or CD43 mAb MEM-257 in PBS. The slides were then blocked with 2% FCS in PBS. Afterwards, the cells were added and incubated at 37 °C for indicated time points in HBSS containing 1 mM Ca^{2+} and 1 mM Mg^{2+}. After stimulation, the cells were fixed with 3.7% paraformaldehyde for 10 min. Residual reactive sites of formaldehyde were blocked with a 0.2% gelatin/1% glycine mixture. Before staining, cells were blocked using 2% human IgG in 2% FCS-PBS. Then, the cells were stained for 30 min at room temperature with mAbs against CD18, CD43 and CD147 labeled with different Alexa Fluor dyes (AF488, AF555, AF647) at a final concentration of 10 μg/ml. After washing twice with 2% FCS-PBS, cells were mounted on glass slides. The samples were analyzed using a Zeiss LSM-510 Meta laser scanning confocal microscope system with 40× magnification.

3. Results

3.1. CD43 interacts with CD147 in human leukocytes

To find potential partners of CD43, we screened a human Jurkat T cell Y2H cDNA library using the intracellular domain of CD43 (amino acids 277–400) as bait. With the exception of Daxx, which was recently described by some of us as a part of the apoptosis pathway of CD43 (Cermak et al., 2002), the only specifically interacting clone was the intracellular part plus two amino acids of the putative transmembrane region of the transmembrane protein CD147 (amino acids 227-269; see Table 1). To confirm the protein-protein interaction of CD43 and CD147 we performed a series of immunoprecipitation experiments using the human myeloid cell line U937, the human T cell line Jurkat as well as the mouse T cell line BW5147 transduced with both human CD43 and human CD147. As shown in Fig. 1, we were able to co-precipitate CD43 with CD147 and vice versa from lysates of these cells. Interestingly, only the high molecular weight form of CD147 (50–66 kDa) co-precipitated with CD43 indicating that the mature glycosylated form of CD147 expressed on the cell surface associated with CD43.

Table 1
Yeast 2 Hybrid screen[a]

Bait plasmid	Prey plasmid	Growth on SD/G/-WH	Growth on SD/G/-WHUL	Growth on SD/GR/-WHUL
pLexA-p53	pB42AD-T	+++	–	+++
pLexA-lamin	pB42AD-T	+++	–	–
pLexA-CD43 (icd)	pB42-CD147(icd)	+++	–	+++
pLexA-lamin	pB42-CD147(icd)	+++	–	–

[a] A human Jurkat T cell leukemia cDNA library cloned in the Y2H vector pB42AD (Clontech) was screened according to the manufacturer's instruction for CD43 interaction partners using the intracellular domain of CD43 (amino acids 277–400) as bait. Bait plasmids containing p53 and lamin were used as controls. Positive clones were selected upon Gal/Raf-induced expression of the cDNA library by using selective growth media. (SD/G/-WH: synthetic dropout agar medium with glucose, lacking tryptophan and histidin; SD/G/-WHUL: synthetic dropout agar medium with glucose, lacking tryptophan, histidin, uracil and leucin; SD/GR/-WHUL: synthetic dropout agar medium with galactose/raffinose, lacking tryptophan, histidin, uracil and leucin).

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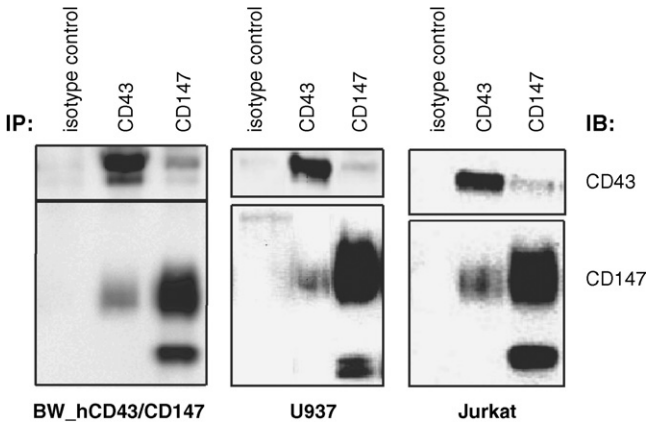

Fig. 1. Co-immunoprecipitation of CD43 and CD147. The CD43 and CD147 molecules were immunoprecipitated (IP) with CD43 mAb MEM-257 and CD147 mAb MEM-M6/3, respectively, from cell lysates of U937, Jurkat and BW5147 mouse thymoma cells. The latter were doubly transfected with human CD43 and human CD147. A mAb against alpha-fetoprotein (AFP-01) was used as an isotype-matched control mAb. Co-immunoprecipitation of CD43 with CD147 was analyzed by immunoblotting (IB) using CD43 mAb MEM-59 and CD147 mAb MEM-M6/3.

3.2. CD43 and CD147 mAbs induce homotypic cell aggregation and adhesion to ICAM-1

Based on the known regulatory role of CD43 and CD147 in cell adhesion we assumed that the interaction of these two transmembrane molecules might be involved in modulation of LFA-1-mediated adhesiveness. We tested different CD43 and CD147 mAbs in cell adhesion assays using myeloid U937 and Jurkat T cells. The CD43 mAbs MEM-59 and MEM-257 and the CD147 mAb MEM-M6/8 induced homotypic cell adhesion in both cell types (Fig. 2A). Cell adhesion induced via CD43 was stronger than via CD147, especially in U937 cells. The CD147 mAb MEM-M6/1 did not show any effect. Inasmuch as this mAb is of the same isotype (IgG1) as the activating mAb, we judged it as a non-functional negative control for this and further experiments. The metabolic inhibitor sodium azide and the cytoskeleton blocking reagent cytochalasin B abrogated the homotypic cell adhesion induced via both CD43 and CD147 mAbs (data not shown) indicating that the observed adhesion is an active process and not a simple agglutination of the cells by the mAbs. Next, we determined whether CD43 and CD147 could modulate LFA-1 activity. We analyzed cell adhesion to plates coated with ICAM-1Rg in the presence of the above-described CD43 and CD147 mAbs. As shown in Fig. 2B, we found a significant induction of adhesion in the presence of the functional mAbs compared to the control mAbs.

3.3. Dynamic co-capping of LFA-1 upon CD43- and CD147-induced cell adhesion

To analyze re/organization of the molecules under study in the cellular environment, we performed confocal microscopy. Jurkat cells were incubated on cover slips coated with the adhesion inducing mAbs to CD43 and CD147. Detection of CD18, CD43 and CD147 was done with specific mAbs labeled with different Alexa Fluor dyes (AF488, AF555, and AF647, respectively). After 5 min of contact with the stimulating CD147 mAb MEM-M6/8, redistribution of both CD18 and CD43 started in the membrane of Jurkat cells (not shown). After 30 min more than 80% of CD18 and CD43 were co-localized in areas of cell-cell contact. However, not all CD147 molecules co-localized: we found a considerable portion outside the cap (Fig. 3A). We observed a similar but not identical phenotype by triggering CD43 with mAb MEM-257: in contrast to the CD147 stimulation, the majority if not all of the CD147 molecules capped in the cell contact zone while only a portion of CD43 and CD18 did so (Fig. 3B). After 1 h of stimulation via both CD43 and CD147 mAbs, CD18 as well as CD43 and CD147 were again equally redistributed on the cell surface (data not shown). This CD43- and CD147-induced dynamic co-capping of CD18, CD43 and CD147 indicated that the three molecules temporarily cooperate.

3.4. CD43 but not CD147 interacts directly with LFA-1

The strong co-capping of CD43 with CD147 (Fig. 3) is consistent with their cytoplasmic interaction (Table 1) and reflected by the co-immunoprecipitation shown in Fig. 1. Because both molecules co-capped with CD18 in focal areas of the cell membrane (Fig. 3), we analyzed a possible direct interaction with CD18 via co-immunoprecipitation. As shown in Fig. 4, we co-precipitated CD43 and CD18 from lysates of U937, Jurkat and primary human T cells. Strikingly, we never found CD147 in the CD18 precipitate and vice versa (Fig. 4 and data not shown). These findings together with the results shown in Fig. 3 indicate that two distinct CD43 complexes exist in the membrane of leukocytes, one containing CD18 the other one CD147.

3.5. CD43 interferes with CD147 mAb-induced homotypic cell aggregation

Although CD18 clustered in cell contact zones stronger upon CD147- than upon CD43 triggering (Fig. 3), cell aggregation and attachment to immobilized ICAM-1 was weaker upon CD147 treatment (Fig. 2). Because this CD147-induced cap contained also the majority of CD43, we assumed that CD43 inhibits LFA-1 by temporal association. If this were true, then CD147-triggering in the absence of CD43 should result in stronger cell adhesion. To study this, we analyzed the mouse thymoma cell line BW5147 retrovirally transduced either with human CD43, human CD147 or both. We selected clones, which expressed the molecules at similar levels (Fig. 5A). The CD147 mAb MEM-M6/8 induced a strong homotypic cell adhesion in the clone transfected with human CD147 alone (Fig. 5B). Adhesion induction was abrogated by adding the metabolic inhibitors sodium azide and sodium fluoride, as well as by cytochalasin B, which blocks the cytoskeleton re-arrangement (Fig. 5C). This indicated that the CD147 mAb-induced homotypic cell aggregation was mediated not only in Jurkat T cells (Fig. 2A) but also in these cells via active signal transduction through CD147 and not

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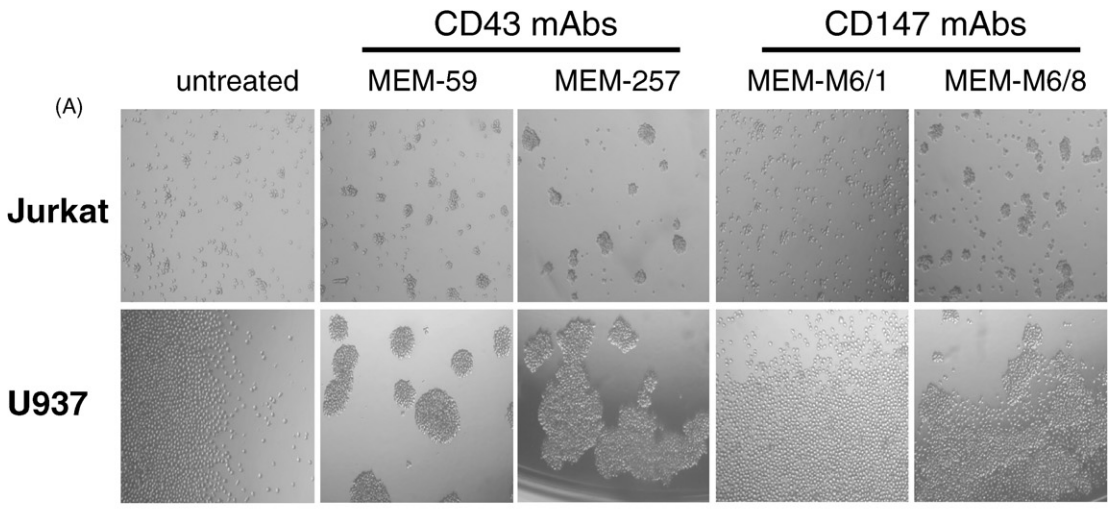

mAbs	Aggregation Score	
	Jurkat	U937
Untreated cell	2	-
MEM-59 (CD43 mAb)	4	>4
MEM-257 (CD43 mAb)	4	>4
MEM-M6/1 (CD147 mAb)	2 to 3	-
MEM-M6/8 (CD147 mAb)	3 to 4	>4

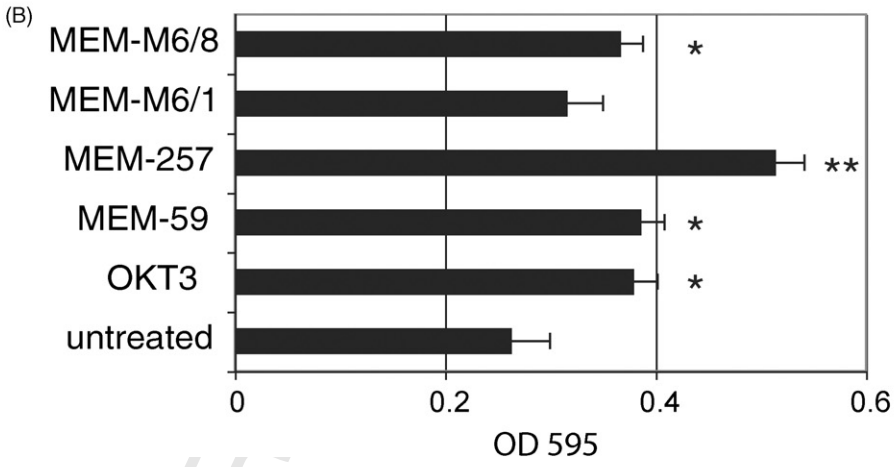

Fig. 2. CD43 and CD147 mAb-induced ICAM-1-mediated homotypic cell adhesion. A, U937 or Jurkat cells were cultured in the presence or absence of 10 μg/ml of CD43 mAbs (MEM-59 or MEM-257) or CD147 mAbs (MEM-M6/1 or MEM-M6/8) at 37 °C. Cell aggregation was scored after 5 hr for Jurkat and 24 hr for U937 cells (n = 3) (see score table). B, Jurkat cells (1×10^5 cells/well) were incubated at 37 °C for 1 h on ICAM-1Rg coated wells (96 well plate) in the presence or absence of the indicated mAbs (final concentration 10 μg/ml). Non-adherent cells were washed away with PBS. The adherent cells were fixed and stained with 0.1% crystal violet and the number of cells was determined as described in Materials and Methods. The CD3 mAb OKT3 was considered as positive control and the non-functional CD147 mAb MEM-M6/1 as negative control. The experiment was repeated 3 times. A probability value below 0.05 was considered significant (* = $p < 0.05$; ** = $p < 0.005$).

by passive agglutination of the mAb. Furthermore, the CD147 mAb-induced cell adhesion was dependent on divalent cations (Fig. 5C), pointing to activation of integrins. Strikingly, co-expression of CD43 inhibited completely the CD147-induced cell adhesion.

We also tested CD43 single transfected cells: as shown in Fig. 5B the CD43 mAb MEM-59 induced a strong cell adhesion. When co-expressed with CD147 in the double-transduced cells, the CD43-induced adhesion was diminished but still remained strong (Fig. 5B). The staining experiment in Fig. 5A also assured that the CD43/CD147 co-expression did not interfere with the binding of the mAbs. These results indicate that the molecular mechanisms underlying cell adhesion via CD43 and CD147 are overlapping and that CD43 is an inhibitor of the latter one.

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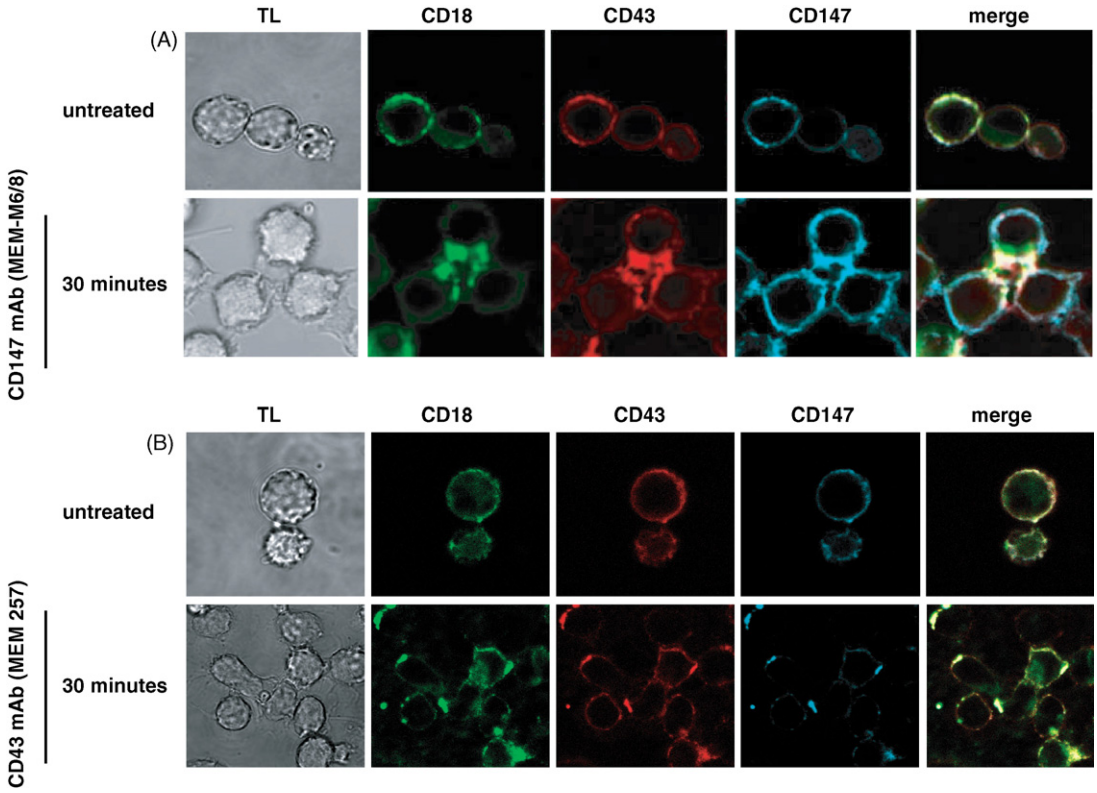

Fig. 3. Dynamic co-capping of CD18, CD43 and CD147. Jurkat cells were triggered on cover slips coated with either (A) CD147 mAb MEM-M6/8 or (B) CD43 mAb MEM-257 at 37 °C for different time points. Afterwards, cells were fixed and stained with Alexa Fluor (AF)-labeled mAbs to CD18 (MEM-48/AF488), CD43 (MEM-59/AF555) and CD147 (MEM-M6/1/AF647). The analysis was performed using an LSM 510 Meta laser scanning microscope system from Zeiss with 40× magnification. In addition to the fluorescence- also a transmitted light (TL) image was recorded.

3.6. CD43 interferes with cell adhesion to ICAM-1 in human T cells

Finally, we tested whether the negative regulatory effect of CD43 on CD147–induced cell adhesion as observed in the mouse transductants is present also in human cells. Therefore, we down-regulated CD43 expression in Jurkat T cells by transfection with small interfering RNAs (siRNAs) (Fig. 6A). Then, we tested the CD43 silenced cells for their ability to adhere to ICAM-1 upon stimulation with the CD147 mAb MEM-M6/8. As shown in Fig. 6B, the CD43 knock down cells adhered stronger to ICAM-1 after treatment with the CD147 mAb when compared to control siRNA transfected cells. Interestingly, also adhesion induced via the T cell receptor by the CD3 mAb OKT-3 was enhanced in the knock down cells. Moreover, the basal adherence to ICAM-1, without any stimulation, was stronger in the CD43 knock down cells.

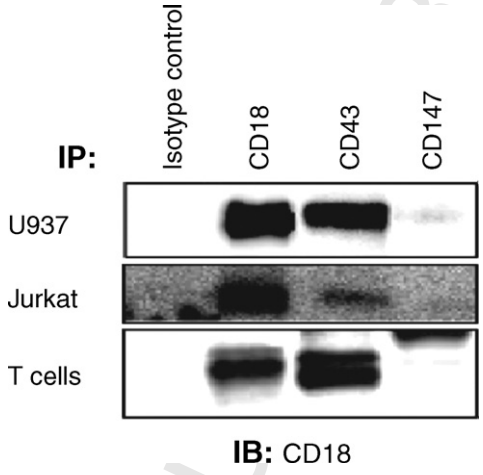

Fig. 4. CD43 interacts directly with LFA-1. Cell lysates of U937-, Jurkat- and primary human T cells were immunoprecipitated (IP) with CD18 mAb MEM-48, CD43 mAb MEM-257 or CD147 mAb MEM-M6/3. The mAb AFP-01 served as negative control. The samples were analyzed by immunoblotting (IB) using the CD18 mAb MEM-48.

4. Discussion

The pro-adhesive and anti-adhesive role of CD43 in the regulation of cell adhesion is the matter of a long term debate (Ostberg et al., 1998; Woodman et al., 1998). A possible mechanism mediating the negative regulatory function of CD43 for leukocyte adhesion is supposed to be steric hindrance of cell interactions via its extended conformation and its negatively charged sialic acid residues (Ostberg et al., 1998) (Allenspach et al., 2001). Our finding that CD43 co-immunoprecipitated with LFA-1 in U937, Jurkat and primary human T cells and negatively regulates CD3 or CD147-induced LFA-1 adhesion is in line with this idea.

The coordinated de-adhesion of integrins is especially important at the rear edge of migrating cells (Friedl and Wolf, 2003).

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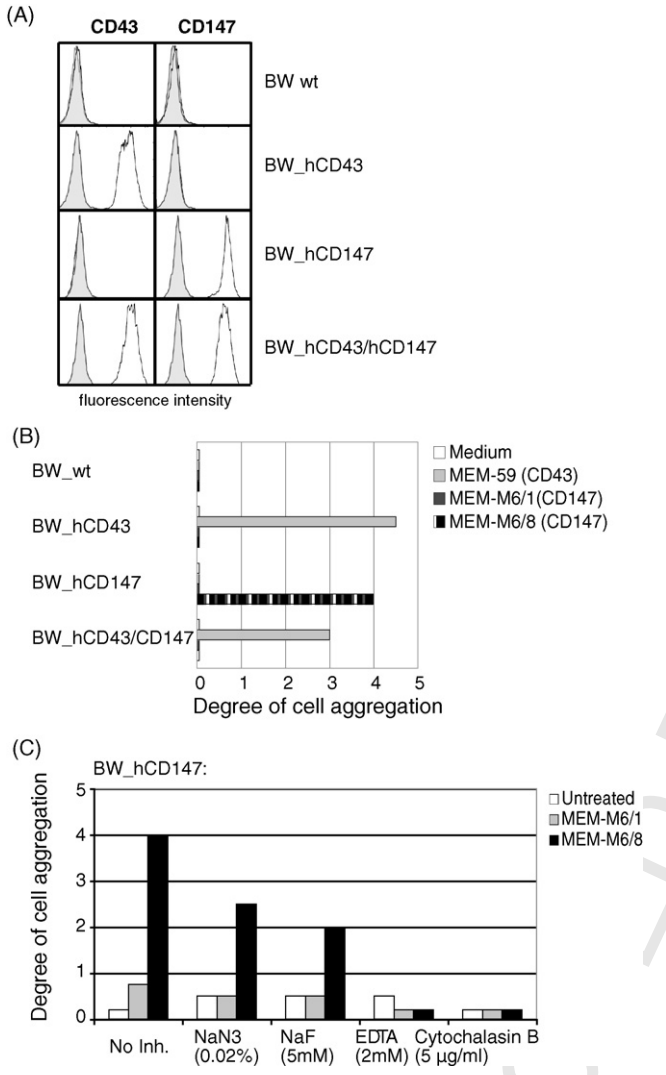

Fig. 5. Induction of cell adhesion via CD147 is inhibited by CD43 expression. A, The mouse T-lymphoma cell line BW5147 was transduced with human CD43 (BW_hCD43), human CD147 (BW_hCD147) or both (BW_hCD43/hCD147). The single and double transductants were analyzed by flow cytometry using CD43 and CD147 mAbs. B, Untransduced wild type (BW-wt) and transduced BW5147 cells were cultured in the presence or absence of the CD43 mAb MEM-59 or the CD147 mAbs MEM-M6/8 or MEM-M6/1 at a final concentration of 10 µg/ml. C, BW_hCD147 cells were pre-incubated with or without 0.02% NaN_3, 5 mM NaF, 2 mM EDTA or 5 µg/ml cytochalasin B for 30 min at 37 °C and thereafter the adhesion-inducing CD147 mAb MEM-M6/8 or the non-functional CD147 mAb MEM-M6/1 was added. Cell aggregation was scored after 24 h by using an inverted microscope. Results are representative of three independent experiments.

CD43 is recruited to the cellular uropod during leukocyte cell migration (Dehghani Zadeh et al., 2003; Sanchez-Mateos et al., 1995) where it could either mediate de-adhesion via its extracellular features and/or via an active signaling event leading to LFA-1 inactivation. CD43 recruitment to the cellular uropod was shown to be mediated by ERM (ezrin/radixin/moesin) cytosceletal adaptor proteins (Allenspach et al., 2001). Savage and colleagues found that CD43 expression at the contact zone between T cells and the matrix blocked the LFA-1-mediated adhesion (Savage et al., 2002). T cell migration on ICAM-1 involves regulation of MLCK-mediated attachment and ROCK-dependent detachment (Smith et al., 2003). As CD43 is also recruited to the trailing edge it could influence these signaling cascades, e.g. by recruitment of signaling components essential for activation of RhoA upstream of ROCK. Further, cell stimulation through CD43 resulted in the recruitment of different signaling proteins, including members of the Src family kinases, Syk, phospholipase Cgamma2, the adapter protein Shc, the guanine nucleotide exchange factor Vav and the CD3 zeta-chain (Cruz-Munoz et al., 2003).

We asked in this study if there are any additional factors influencing the membrane localization and functions of CD43. Now we can report the identification of a novel interaction partner of CD43 in the type I transmembrane receptor CD147. We identified CD147 as an interacting molecule with CD43 by the Y2H system. We confirmed the Y2H interaction by co-immunoprecipitations in various cell types as well as by co-localization using confocal microscopy. In addition, we provide a series of cell adhesion and aggregation assays indicating that these two molecules do interact not only physically but also cooperate functionally: First, mAbs to both CD43 and CD147 induced homotypic cell aggregation and adhesion to the LFA-1 ligand ICAM-1, second, both CD43 and CD147 mAbs induced co-capping of LFA-1 with CD43 and CD147 molecules at cell contact zones, and third, CD43 interfered with CD147-triggered cell adhesion and aggregation. Strikingly, in contrast to CD43, we were not able to co-immunoprecipitate LFA-1 with CD147 indicating that CD43 interacts with CD147 and LFA-1 in two distinct complexes.

CD147 was already described to interact with beta-1 integrins (Berditchevski et al., 1997; Cho et al., 2001) and to co-localize with actin and integrins, thereby affecting cellular architecture (Curtin et al., 2005). Further, CD147 mAbs were shown to induce homotypic cell aggregation via LFA-1/ICAM-1 interactions (Kasinrerk et al., 1999). Besides the integrin interaction, CD147 forms a supramolecular complex with CD98 (the heavy chain of several types of amino acid transporters) (Xu and Hemler, 2005) and the monocarboxylate transporters MCT1 and MCT4 (Kirk et al., 2000), and plays a regulatory role in the function of these transporters. CD147 is also a receptor for cyclophilins (Pushkarsky et al., 2001; Yurchenko et al., 2001), which are potent chemoattractants detected on the surface of a variety of cells and at high levels in inflamed tissues (Damsker et al., 2007). Recently it has been shown that CD147 regulates the expression levels of matrix metalloproteinases, those of urokinase plasminogen activator (uPA), uPA receptor, plasminogen activator inhibitor-1 (PAI-1) (Quemener et al., 2007) as well as focal adhesion kinase resulting in altered cell motility and invasion (Xu et al., 2007). These data show that CD147 is a key molecule in cell adhesion and migration. In addition, our results demonstrate dynamic association with CD43 and that triggering of either CD43 or CD147 results in their different recruitment with LFA-1 to the cell contact zone (Fig. 3). Therefore, we suggest that different engagement of CD43 and CD147 by the microenvironment might result in different cellular response programs for cell attachment and motility. This assumption is corroborated by the siRNA-mediated knock down of CD43 in human T cells that caused enhanced LFA-1 activa-

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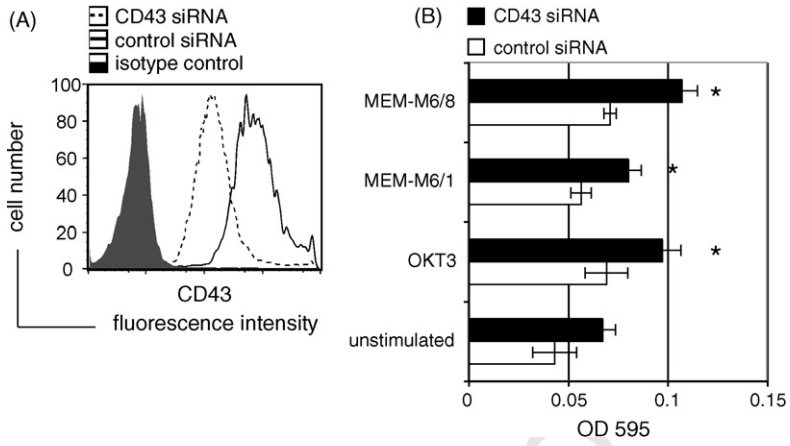

Fig. 6. CD43 interferes with T cell adhesion to ICAM-1. A, Jurkat cells were transiently transfected using either siRNA against CD43 or control siRNA by the Amaxa nucleofection technology. Silencing efficiency was tested by flow cytometry (CD43 siRNA - dashed line, control siRNA - solid line). B, The transfected Jurkat cells were cultured on plates coated with ICAM-1Rg in the presence or absence of CD3 mAb OKT3 or the CD147 mAbs MEM-M6/1 and MEM-M6/8. The number of adherent cells was determined after 1 h incubation by staining with 0.1% crystal violet. The standard deviations were calculated from 12 different wells of 3 independent experiments (4 wells per experiment). An asterisk indicates all values that differ significantly from the control siRNA transfected cells ($p < 0.05$).

tion via CD147 and the T cell antigen receptor (Fig. 6). Thus the dynamic interaction of CD43 with CD147 could be responsible for the anti- and pro-adhesive properties of CD43 towards LFA-1 and explain its role in tuning cell attachment and detachment in immunity.

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