LFA-1 (CD11a/CD18) is a cell adhesion molecule that mediates leukocyte adhesion by binding to one of its ligands: ICAM-1, ICAM-2 or ICAM-3. Here, we investigated whether stimuli known to induce adhesion to ICAM-1 were also capable of inducing LFA-1-mediated adhesion of T lymphocytes to ICAM-2 and -3 transfectants. We observed that phorbol 12-myristate 13-acetate, Mn2+, cross-linking of CD3 or activating antibodies against LFA-1 enhanced LFA-1-mediated T cell adhesion to ICAM-2 and -3, although to a lesser extent than to ICAM-1. These results indicate that, similar to what has been reported for adhesion to ICAM-1, activation of LFA-1 is also required for adhesion to ICAM-2 and -3. Furthermore, the results suggest that ICAM-1 is the major ligand for LFA-1 on activated T lymphocytes. Interestingly, we observed that in contrast to activating antibodies against CD18, activating antibodies against CD11a were incapable of inducing adhesion of LFA-1 to all three ligands. The antibody MEM-83 stimulated binding to ICAM-1, while at the same time inhibiting the interaction of LFA-1 with ICAM-2 and -3. The antibody NKI-L16 selectively induced adhesion to ICAM-1 and -2, but not to ICAM-3. Our results suggest that different conformations of LFA-1 are required to support adhesion to ICAM-1, -2 or -3, and that ligands may bind on different sites of the LFA-1 molecule.

1 Introduction

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1 Introduction

LFA-1 (CD11a/CD18) is a cell adhesion molecule that is involved in a broad range of immunological processes [1, 2]. LFA-1 belongs to the β2-(CD18) family of integrins, which consist of an α-chain and a β-chain which are non-covalently linked [3]. Thus far three ligands for LFA-1 have been described: ICAM-1 [4], ICAM-2 [5] or ICAM-3 [6-9], which are all members of the Ig-superfamily and have 5, 2 and 5 Ig-like domains, respectively [5-8, 10-12]. ICAM-1 is expressed on many cell types including lymphocytes and certain epithelial cells. ICAM-1 is strongly up-regulated on resting lymphocytes [13] and is, therefore, important for the migration of leukocytes through endothelial cell layers into inflamed tissue [14]. ICAM-2 is mainly expressed on vascular endothelium and some lymphoid cells. In contrast to ICAM-1, ICAM-2 is not induced by cytokines [5, 15, 16]. ICAM-2 is the predominant ligand for LFA-1 on resting endothelial cells, and has, therefore, been suggested to be important for recirculation of resting lymphocytes [15]. In contrast to ICAM-1 and -2, ICAM-3 is only expressed on leukocytes with the exception of some endothelial cells in tumors [17, 18]. Since ICAM-3 is the most abundantly expressed ICAM on resting lymphocytes it has been proposed to be the major ligand for LFA-1 during the initiation of immune responses. This hypothesis is supported by the finding that resting lymphocytes adhere to purified LFA-1 predominantly through ICAM-3 [9]. Recent reports suggest that ICAM-3 serves not only as an adhesion receptor, but is also involved in signal transduction [19, 20]. ICAM-3 is a co-stimulatory molecule for both resting and activated T cells [19]. Furthermore, it was shown that cross-linking of ICAM-3 by anti-ICAM-3 mAb increases the avidity of LFA-1 for ICAM-3 [20].

Considerable information has been gathered about the regulation of LFA-1-mediated adhesion to ICAM-1. LFA-1 does not mediate stable adhesion to ICAM-1 unless activated [21-23]. This activation can be induced by different stimuli and requires a physiological temperature, an intact cytoskeleton and the presence of divalent cations [24]. Cross-linking of certain surface receptors on T lymphocytes, such as CD2 and CD3 [22, 23] or addition of the phorbol ester PMA [25, 26] generate intracellular signals that increase the avidity of LFA-1 for ICAM-1. In addition, divalent cations such as Mn2+ have been shown to stimulate LFA-1-mediated adhesion [27]. Enhanced LFA-1-mediated binding to ICAM-1 is furthermore observed upon binding of certain unique activating antibodies directed against the LFA-1 α- or β-chain. Since Fab fragments of most of these antibodies induce adhesion, activation is not caused by cross-linking but is most likely due to the induction of a conformational change within the molecule [28-32]. Thus far, five LFA-1 activating antibodies have been described, which all bind to distinct epitopes [van Kooyk et al., submitted]. NKI-L16 [28, 29] and MEM-83 [30] recognize epitopes on the LFA-1 α-chain (CD11a), while KIM127 [31], KIM185 [32] and MEM-48 (van Kooyk et al., submitted) are directed against the common β2-chain (CD18).
Since little is known about requirements for adhesion of LFA-1 to ICAM-2 and -3, we here investigated whether stimuli known to induce LFA-1-mediated adhesion to ICAM-1 were also capable of stimulating binding of T lymphocytes to ICAM-2 and -3.

2 Materials and methods

2.1 mAb and chemicals

The following mAb were used: NKI-L15 (IgG2A) [33], NKI-L16 (IgG2A) [28, 29] and MEM-83 (IgG1) [30] directed against the α-chain of LFA-1 (CD11a), MEM-48 (IgG1) (van Kooyk et al., submitted), KIM127 (IgG1) [31] and KIM185 (IgG1) [32] reactive with the LFA-1 β-chain (CD18); mAb CBR-IC2/2 (IgG2A) reactive with ICAM-2 (CD102) [15], mAb CBR-IC3/1 (IgG1) [9] and mAb CBR-IC3/2 (IgG2A) [8] reactive with ICAM-3 (CD50); mAb CLB-T3/4E (IgE) [34] reactive with CD3; RR1/1 (IgGl) reactive with ICAM-1. MAb REK-1 was obtained by immunizing BALB/c mice with a CTL clone. Subsequently hybridoma supernatants were screened for reactivity with ICAM-1 transfectants (CD54). All mAb were used at a concentration of 5–10 μg/ml.

Reagents used were PMA (50 ng/ml; Sigma Chemical Co, St. Louis, MO) and MnCl2 (10 mM; diluted in Hepes/NaCl buffer). 

2.2 Cells

The T cell clone JS136 [35] was cultured as described previously [29]. Mouse L cells expressing ICAM-1 under control of the CMVAD169 immediate early promoter, were obtained as described [36]. L cells and L cell transfectants were cultured in Iscove’s medium with L-glutamine (Gibco, Gaithersburg, MD). L cells and L cell transfectants expressing high amounts of human ICAM-2 or ICAM-3 were obtained following selection in medium containing 500 μg/ml hygromycin B (Calbiochem, San Diego, CA) and subsequent positive sorting on a FACStar (Becton Dickinson).

2.5 Adhesion assay

Monolayers of L cells transfected with ICAM-1, -2 or -3 and non-transfected L cells were grown in 96-well tissue culture plates. In these wells Na235CrO4-labeled T lymphocytes were incubated with PMA (50 ng/ml), Mn2+ (1 mM) or with blocking or activating antibodies (5–10 μg/ml) during 10 min at room temperature, cells were centrifuged at 400 rpm for 1 min and were subsequently incubated at 37°C during 30 min. Non-adherent cells were washed away with warm medium, adherent cells were lysed with 1% Triton X-100 and radioactivity was quantified. Results are expressed as the mean per cent of cells binding from triplicate wells.

3 Results

3.1 Distinct binding of T lymphocytes to ICAM-1, -2 or -3 transfectants upon activation of LFA-1

To investigate the regulation of T lymphocyte adhesion to ICAM-1, -2 and -3, we generated stable mouse L cell transfectants expressing high levels of ICAM-1, -2 or ICAM-3 (Fig. 1). ICAM-1, -2 or -3 expressed on these cells is functional, since the LFA-1 activating antibody KIM185 (CD18) induced adhesion of the T cell clone JS136 to either one of these transfectants. The T cell clone JS136 was selected for experiments, because of its high expression of LFA-1 on the cell surface [29]. Adhesion was specific since it could be blocked by antibodies directed against LFA-1 or by antibodies directed against ICAM-1, -2 and -3, respectively (Fig. 2).
LFA-1-mediated T lymphocyte adhesion to ICAM-1, -2 and -3

Next we determined whether various stimuli known to activate the LFA-1/ICAM-1 interaction were also capable of inducing LFA-1-mediated binding of the T cell clone JS136 to ICAM-2 or -3 transfectants (Fig. 3). We observed that Mn²⁺, PMA and cross-linking of CD3 increased adhesion of JS136 T cells to ICAM-2 and -3, however to a lesser extent than adhesion to ICAM-1. LFA-1-mediated adhesion of JS136 could also be induced by LFA-1 activating antibodies. Interestingly, we observed differences in the capacity of the various antibodies to induce adhesion to ICAM-1, -2 or -3. Activating antibodies directed against the LFA-1 β-chain induced adhesion to all three ligands. KIM185 was most effective and strongly increased adhesion to ICAM-1, -2 and -3, while KIM127 increased adhesion to ICAM-1 to a higher extent than adhesion to ICAM-2 and -3. MEM-48 strongly increased adhesion to ICAM-1 and to a lesser extent to ICAM-2, while only weak binding to ICAM-3 was observed. In contrast to the activating antibodies against the LFA-1 α-chain, activating antibodies directed against the α-chain were not capable of stimulating adhesion to all three ligands. NKI-L16 induced adhesion to ICAM-1 and -2 but not to ICAM-3, while MEM-83 stimulated binding to only ICAM-1.

3.2 MEM-83 induces LFA-1-mediated T cell adhesion to ICAM-1, but inhibits binding to ICAM-2 and ICAM-3

In an attempt to explain the observation that MEM-83 only induced LFA-1 mediated T cell adhesion to ICAM-1, we investigated whether MEM-83 interfered with binding of LFA-1 to ICAM-2 and -3 (Fig. 4). To this end we combined stimuli that induced adhesion of JS136 T cells to all three ligands with the MEM-83 antibody. We observed that LFA-1 mediated T cell adhesion to ICAM-2 induced by KIM185 was blocked to background levels by MEM-83, whereas adhesion induced by KIM127 or PMA was only
Figure 4. Inhibition of LFA-1 mediated adhesion of JS136 T cells to L-ICAM-2 and -3 transfectants by the activating antibody MEM-83 (CD11a). The number of adherent JS136 T cells on monolayers of L-ICAM-1, -2 or -3 transfectants was determined as described in Fig. 2, in the presence of medium alone (□), the activating antibodies KIM185 or KIM127 (CD18; '10 ng/ml), PMA (50 ng/ml) or in the presence of a combination of these stimuli with MEM-83 (10 μg/ml) (■). Data are representative of three experiments.

3.3 NKI-L16 stimulates LFA-1-mediated T cell adhesion to ICAM-1 and -2, but not to ICAM-3

Since NKI-L16, like MEM-83, did not induce LFA-1-mediated T cell adhesion to ICAM-3, we investigated whether NKI-L16 inhibited binding of LFA-1 to ICAM-3 (Fig. 5). We observed that in contrast to MEM-83, adhesion induced by KIM185, KIM127 or PMA was unaffected by the NKI-L16 antibody. These results indicate that the inability of NKI-L16 to induce adhesion to ICAM-3 is not due to inhibition of the interaction of LFA-1 with ICAM-3.

4 Discussion

Using stable transfectants expressing ICAM-1, -2 or -3 cDNA we investigated the capacity of LFA-1 to bind to each of these ligands. The results show that (1) similar to what has been reported for ICAM-1, activation of LFA-1 is also required for adhesion to ICAM-2 and -3. (2) Activation of LFA-1 yields high T cell adhesion to ICAM-1, intermediate adhesion to ICAM-2, and low adhesion to ICAM-3, suggesting that ICAM-1 is the major ligand for LFA-1 on activated T lymphocytes. (3) Adhesion to ICAM-1 and ICAM-2 can be induced by activating antibodies directed against the LFA-1 α- or β-chain, while binding to ICAM-3 is only induced by activating antibodies against the LFA-1 β-chain. (4) MEM-83 induces adhesion to only ICAM-1, since it blocks the interaction of LFA-1 with ICAM-2 and -3. (5) NKI-L16 induces adhesion to only ICAM-1 and -2 and not to ICAM-3; however, it does not inhibit binding of LFA-1 to ICAM-3.

The results obtained with MEM-83 suggest that this antibody is able to enforce a conformational change on LFA-1 that enhances adhesion to ICAM-1, but renders LFA-1 inaccessible to ICAM-2 and ICAM-3. This may imply that ICAM-1 binds to a site in the LFA-1 molecule which is distinct from the binding sites for ICAM-2 and -3.
MEM-83 maps to the I domain, supporting the hypothesis that the I domain is involved in binding of LFA-1 to its ligands [38-41]. However, the observation that MEM-83 is unable to induce adhesion to ICAM-2 and -3, does not exclude that the I domain is involved in binding of LFA-1 to ICAM-2 or -3, since it is still possible that binding site for ICAM-2 and -3, when located in the I domain, are stericly hindered by MEM-83. Studies using purified I domain fragments will be required to elucidate whether the I domain is involved in binding of LFA-1 to ICAM-2 and -3. In contrast to MEM-83, the incapacity of NKI-L16 to induce adhesion to ICAM-3 was not due to inhibition of the interaction of LFA-1 with ICAM-3. These results, therefore, suggest that LFA-1 can acquire a conformation that selectively binds to ICAM-1 and -2, but not to ICAM-3.

From these data it is tempting to speculate that binding of LFA-1 to its different ligands may involve exposition of ligand-specific binding sites or induction of ligand-specific conformational changes in LFA-1. This notion is supported by the observation that adhesion of β1-integrins to their different ligands may also involve distinct conformational changes in integrin molecules. The β1-integrin activating antibody TS2/16 (CD29) induced stronger adhesion of VLA-4 to fibronectin than to VCAM-1, under suboptimal conditions [42]. In addition the activating antibody TASC (CD29) inhibited adhesion of β1-integrins to vitronectin, while it promoted adhesion to laminin and collagen [43]. However, our results do not exclude the possibility that the incapacity of NKI-L16 and MEM-83 to induce adhesion to ICAM-3 and ICAM-2 and -3, respectively, may be due to differences in the glycosylation of ICAM-1, -2 and -3 molecules. Interestingly, ICAM-2 and ICAM-3 have three and five possible N-linked glycosylation sites in the first Ig-like domain, respectively, whereas ICAM-1 lacks glycosylation in its first domain [5-8, 11]. N-linked glycosylation in the first Ig-like domain may interfere with binding of ICAM-2 and -3 to the conformations of LFA-1 induced by MEM-83 or NKI-L16.

In contrast to the activating antibodies directed against the LFA-1 β-chain, PMA or cross-linking of CD3 only slightly increased adhesion to ICAM-3, suggesting that activation of LFA-1 through intracellular signals does not result in strong binding of activated T cells to ICAM-3. However, it should be noted that an ever increasing number of cell surface receptors expressed by T cells has been implicated in activation of LFA-1 [44-49]. It is, therefore, possible that adhesion of LFA-1 to ICAM-3 requires intracellular signals generated upon triggering of surface receptors distinct from CD3. In contrast to our results, Campanero et al. [20] observed high adhesion of T cells to ICAM-3 purified from neutrophils, upon stimulation with PMA, NKI-L16 or CD3 cross-linking. A possible explanation for these different results might be that ICAM-3 transfected into murine fibroblasts is differently glycosylated than ICAM-3 on neutrophils, and therefore less effective in supporting adhesion of LFA-1. However, the observation that strong adhesion to ICAM-3 transfecants was induced by activating antibodies against CD18, argues against this possibility. Furthermore, immunoprecipitation of ICAM-3 from L-ICAM-3 transfected cells revealed a 128-kDa protein (not shown), which is comparable to the size of ICAM-3 on neutrophils (124 kDa). Since ICAM-3 on neutrophils is 135 kDa in size [17], this indicates that ICAM-3 molecules on L-ICAM-3 transfecants do not differ largely in overall glycosylation from ICAM-3 molecules on neutrophils. Finally, LFA-1-mediated adhesion to purified ICAM-3 molecules may differ from adhesion to ICAM-3 transfecants. It has been shown that LFA-1 expressed on leukocytes requires activation to bind to ICAM-1, while purified LFA-1 molecules are constitutively active [9, 10, 21-23], suggesting that coating of the purified receptors induces a conformational change in LFA-1. Similarly, coated, purified ICAM-3 molecules may be more capable of supporting adhesion than ICAM-3 molecules imbedded in the cell membrane. Therefore, direct comparison of the different models is required to determine which model most closely represents physiological conditions.

Conformational changes in LFA-1 may determine the ligand specificity of LFA-1, however differential expression of ligands on different cell types is another mechanism to regulate LFA-1-mediated adhesion. ICAM-3 is the most abundantly expressed ligand on resting lymphocytes and has, therefore, been proposed to be an important ligand for LFA-1 during the initiation of immune responses. This hypothesis is supported by the finding that resting lymphocytes bind to purified LFA-1 predominantly through ICAM-3 [9]. Purified LFA-1 molecules are constitutively active, supporting our finding that activation of LFA-1 is required for adhesion to ICAM-3. We showed that LFA-1 on resting lymphocytes can hardly be activated to bind to ICAM-1 [50], ICAM-2 or -3 (unpublished observations). Therefore, these results imply that during the initiation of immune responses ICAM-3 on resting lymphocytes has to bind to LFA-1 on more activated cells. These cells might be antigen-presenting cells, but possibly also other cell types are involved. To get more insight into the role of LFA-1/ICAM-3 in the initiation of immune responses, identification of these cells is, therefore, of major importance.

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5 References
