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Analysis of the Molecular Event of ICAM-1 Interaction with LFA-1 During Leukocyte Adhesion Using a Reconstituted Mammalian Cell Expression Model

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Ligand-receptor clustering event is the most important step in leukocyte adhesion and spreading on endothelial cells. Intercellular adhesion molecule-1 (ICAM-1) has been shown to enhance leukocyte adhesion, but the molecular event during the process of adhesion is unclear. To visualize the dynamics of ICAM-1 movement during adhesion, we have engineered stable Chinese hamster ovary cell lines expressing ICAM-1 fused to a green fluorescent protein (IC1_GFP/CHO) and examined them under the fluorescence microscopy. The transfection of IC1_GFP alone in these cells was sufficient to support the adhesion of K562 cells that express αLβ2 (LFA-1) integrin stimulated by CBR LFA-1/2 mAb. This phenomenon was mediated by ICAM-1-LFA-1 interactions, as an mAb that specifically inhibits ICAM-1-LFA-1 interaction (RR1/1) completely abolished the adhesion of LFA-1$^+$ cells to IC1_GFP/CHO cells. We found that the characteristic of adhesion was followed almost immediately (~10 min) by the rapid accumulation of ICAM-1 on CHO cells at a tight interface between the two cells. Interestingly, IC1_GFP/CHO cells projected plasma membrane and encircled approximately half surface of LFA-1+ cells, as defined by confocal microscopy. This unusual phenomenon was also confirmed on HUVEC after adhesion of LFA-1$^+$ cells. Neither cytochalasin D nor 2,3-butanedione 2-monoxime an inhibitor of myosin light chain kinase blocked LFA-1-mediated ICAM-1 clustering, indicating that actin cytoskeleton and myosin-dependent contractility are not necessary for ICAM-1 clustering. Taken together, we suggest that leukocyte adhesion to endothelial cells induces specialized form of ICAM-1 clustering that is distinct from immunological synapse mediated by T cell interaction with antigen presenting cells.

The adhesion of leukocytes to the vascular endothelial lining and their subsequent diapedesis constitute one of the earliest changes detectable in inflammation, immune responses, and atherosclerosis (Luscinskas et al., 1996; McEvoy et al., 1997; O'Brien et al., 1996; Raines and Ross, 1996). Leukocyte adhesion to endothelial cells can be significantly up-regulated by activating the endothelium with inflammatory cytokines such as tumor necrosis factor α (TNF-α), interleukin-1 (IL-1), and IL-4 (Raines and Ross, 1996). Activated endothelial cells express several monocyte-binding proteins including intercellular adhesion molecule-1 (ICAM-1), ICAM-2, and vascular cell adhesion molecule-1 (VCAM-1) as well as members of the selectin family of adhesion molecules E- and P-selectins (Bevilacqua and Nelson, 1993; Yoshida et al., 1996).

ICAM-1 consists of five extracellular Ig-like domains (D1-D5), a hydrophobic transmembrane domain, and a short cytoplasmic domain (Diamond and Springer, 1994; Diamond et al., 1990). It is a ligand for at least 2 members of the β2 family of leukocyte integrins (LFA-1 and Mac-1) (Diamond and Springer, 1994; Diamond et al., 1990). ICAM-1 is required for stable adhesion, subsequent spreading, and diapedesis of leukocytes through endothelium (Bevilacqua and Nelson, 1993; Dustin et al., 1996). Antibodies to ICAM-1 inhibit leukocyte adhesion to endothelial cells, granulocyte migration through endothelium, mitogen

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and Ag-induced lymphocyte proliferation and mixed lymphocyte reactions (Smith et al., 1989; Smith et al., 1988). Although ICAM-1-LFA-1 interaction at a fundamental level has been studied extensively (Jun et al., 2001; Shimaoka et al., 2001), the kinetics and pattern of ICAM-1 accumulation between endothelial cells and leukocytes are still poorly understood.

The molecular dynamics of T cell recognition to antigen-presenting cells (APC) are well-described (Grakoui et al., 1999). Initial views of antigen-specific T cell junctions have revealed the formation of a specialized contact, termed the immunological synapse (Monks et al., 1998; Paul and Seder, 1994). It consists of a central cluster of T cell receptors surrounded by a ring of adhesion molecules. Among the most significant of these is the binding of the integrin LFA-1 on a T cell to ICAM-1 on the APC (Dustin and Springer, 1989; Grakoui et al., 1999; van Kooyk et al., 1989). Binding of ICAM-1 to LFA-1 is crucial for T cell activation through stabilization of calcium signal at low peptide/MHC concentrations (Wulfing et al., 1998). For several aspects, however, the molecular events of leukocyte transmigration through the endothelial barrier are quite distinct from T cell recognition to APC. First, it needs a stepwise adhesion cascade coordinated by the sequential ligand recognition of cell adhesion molecules expressed on leukocytes and endothelium (Carlos and Harlan, 1994). Second, it does not require specialized synapses as seen in T cell junction. Third, it requires rearrangement of adhesion molecules on endothelium along with leukocyte movement toward the inflammatory region.

In this paper, we present an experimental system that allows one to simultaneously follow morphological changes and distribution of ICAM-1 on live cells in a real time. We have used this approach to study the structural and geometrical rearrangement of ICAM-1/LFA-1 during leukocyte adhesion on ICAM-1 bearing cells. By using a green fluorescent protein (GFP) fusion construct, we were able to follow the distribution of ICAM-1. Unlike T/B cell interface, ICAM-1 bearing cells (ICAM-1/GFP on CHO cells or HUVEC) projected plasma membrane and encircled approximately half surface of LFA-1^+ cells. These data suggest a model of leukocyte adhesion prior to the transmigration through endothelium toward the inflammatory region.

Materials and Methods

Cells and antibodies

Chinese hamster ovary (CHO) cells were grown in RPMI 1640 (Life Technologies) supplemented with 10% FBS, non-essential amino acids (Life Technologies) and 50 μg/ml of gentamicin. HUVEC were purchased from American Type Culture Collection (ATCC) and maintained in medium 199 modified Earle's salt solution containing 20% FBS, 100 μg/ml endothelial growth supplement, 1% Nutridoma NS, and 100 μg/ml heparin. Cells were cultured at 37°C in humidified air containing 5% CO_2. Cells were used between 3 and 7 passages. K562 cells, which stably express LFA-1 were maintained in RPMI 1640 supplemented with 10% FBS, non-essential amino acids (Life Technologies), 50 μg/ml of gentamicin, and 3 μg/ml hygromycin (Shimaoka et al., 2001). mAbs RR1/1 (Miller et al., 1995) and CBR LFA1/2 (Shimaoka et al., 2001) have been previously described. For some experiments, mAbs were directly conjugated with TRITC or Cy3 according to the manufacture's instruction (Promega).

cDNA constructions

To generate ICAM-1/GFP fusion protein, the stop codon of ICAM-1 was replaced with *Sac*I restriction site by a PCR protocol. After PCR, amplified product was digested with *Hind*III and *Sac*I and sub-cloned into the pEGFP-N1 vector (Clonetech), thus giving rise to the mutant plasmid IC1_GFP/pEGFP-N1. The amino acid sequences of a linker polypeptide between full-length ICAM-1 protein and EGFP residues were PRARNPPVAT.

cDNA transfections

ICAM-1/GFP protein was expressed stably in CHO cells using FuGENE™ 6 transfection reagent according to the manufacturer's instructions (Boehringer Mannheim), followed by selection with 1 mg/ml of G418 beginning at 48 h post-transfection. Stable cell line (IC1_GFP/CHO) was maintained in complete medium supplemented with same concentrations of antibiotic.

Flow cytometry analysis

Single-cell suspensions obtained after EDTA (10 mM/PBS) treatment were centrifuged (400 x g, 5 min) and resuspended in PBS containing 1% FBS. Green fluorescence (GFP) was analyzed and quantified on a Becton Dickson FACScan (Mountain View).

Fluorescence microscopy and confocal imaging

For fluorescence microscopy, IC1_GFP/CHO cells (1 x 10^5 cells/coverslip) were grown on glass coverslips and incubated with LFA-1^+ K562 cells (5 x 10^5 cells/coverslip). After 20 min of incubation, cells were washed, fixed with 2% paraformaldehyde, and processed for fluorescence microscopy. For confocal imaging, slides of IC1_GFP/CHO cells were prepared as described above, and then the cells were imaged using Zeiss confocal microscope (Zeiss). Cross-sectional reconstruction was accomplished with a Metamorph program (Princeton Instruments) to combine the entire Z series into a stack projection and to overlay and process final image.

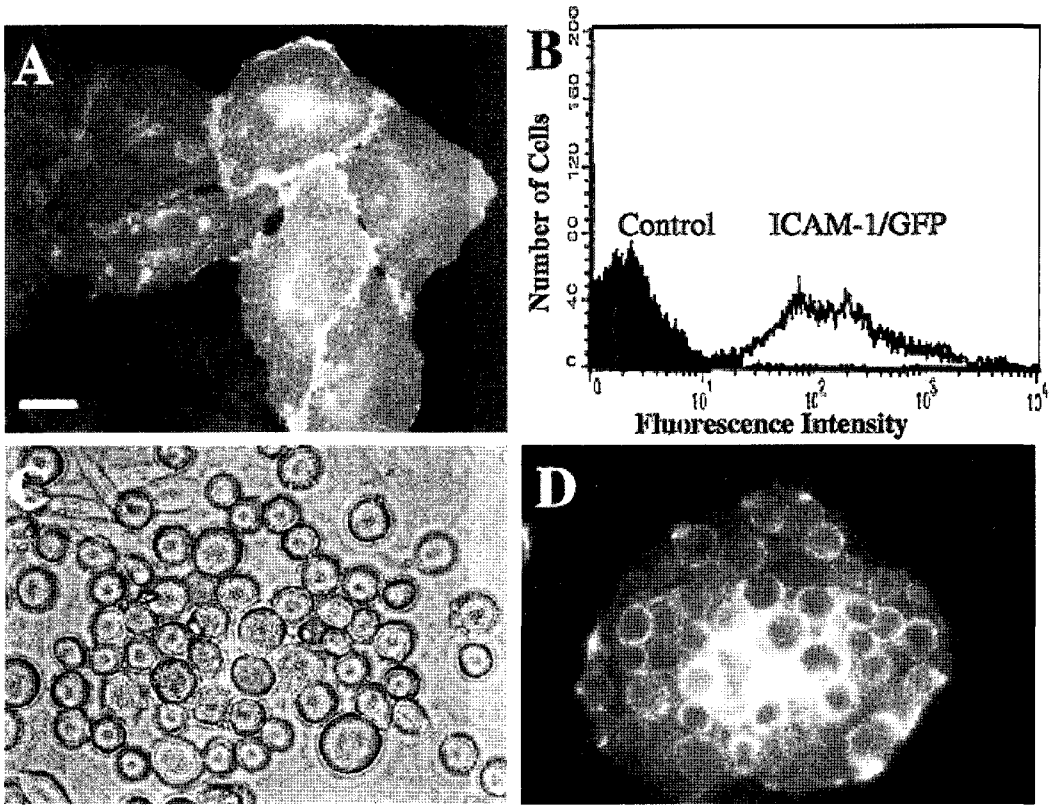

Fig. 1. Characterization of IC1_GFP/CHO cells. A, CHO cells were transfected with IC1_GFP/neo cDNA to generate stable cell line expressing ICAM-1/GFP. Distribution of ICAM-1/GFP was examined under the fluorescence microscopy. B, IC1_GFP/CHO cells were sorted by a magnetic cell sorting system (MACS) by using RR1/1 mAb and the expression of ICAM-1/GFP was quantified by FACScan analysis. C and D, Functional assessment of ICAM-1/GFP. LFA-1$^+$ K562 cells were added to IC1_ GFP/CHO cell monolayers in the presence or absence of LFA1/2 mAb (10 μg/ml). After 20 min of incubation, unbound cells were then removed and the adherent cells were evaluated by microscopy (C). Clustering of ICAM-1/GFP was also evaluated by fluorescence microscopy (D). Bar=10 μm.

Cell adhesion assay

Cell adhesion assay was carried out as described previously by partial modification (Miller et al., 1995). LFA-1$^+$ K562 cells were labeled with fluorescence using a solution of 5 μM BCECF-AM in L15 media supplemented with 5% FBS (L15/FBS). The cells were incubated with BCECF-AM for 45 min in a 5% CO_2 incubator with periodic agitation after which they were washed four times with L15/FBS medium. IC1_GFP/CHO cells were plated in 96-well plates at 1 x 10^4 cells/well and allowed to grow to 2-4 x 10^4 cells/well. The cell layers were washed twice with assay media before adding blocking antibodies at 10 μg/ml. BCECF-labeled cells (1 x 10^5) were pre-incubated with 10 μg/ml of LFA1/2 mAb and added in 50 μl of assay medium. The plates were centrifuged 5 min at 10 x g to bring the BCECF-labeled cells into contact with the monolayer and then incubated for 30 min at 37℃ by flotation in a water bath. Unbound cells were then removed using Microplate Autowasher (Bio-Tek Instruments, Winooski, VT). The fluorescence signal of bound cells (after washing) was expressed as a percentage of the fluorescence of total input cells (before washing) as quantitated on a fluorescent concentration analyzer (IDEXX, Westbrook, ME). The washing procedure was programmed such that binding of mock-transfected cells or binding in the presence of ICAM-1 blocking mAb was below 5% of total input.

Results

Recombinant CHO-K1 cells expressing ICAM-1/GFP

We have developed stable CHO cell lines expressing ICAM-1/GFP. GFP was fused to the ICAM-1 C-terminus with a flexible 10-aa linker (IC1_GFP/CHO). As expected, wild-type CHO (WT-CHO) did not express ICAM-1, whereas IC1_GFP/CHO expressed a high level of ICAM-1 (Fig. 1A). ICAM-1/GFP was uniformly distributed on the upper cell surface, including the cell junctions of the recombinant cell line. Expression level of ICAM-1/GFP was also quantified by FACScan analysis (Fig. 1B). The ICAM-1/GFP expression level was adjusted to match the endogenous ICAM-1 expression level of HUVEC after stimulation with IL-1β (data not shown). To verify whether ICAM-1/GFP is functional in mediating LFA-1-dependent adhesion, we

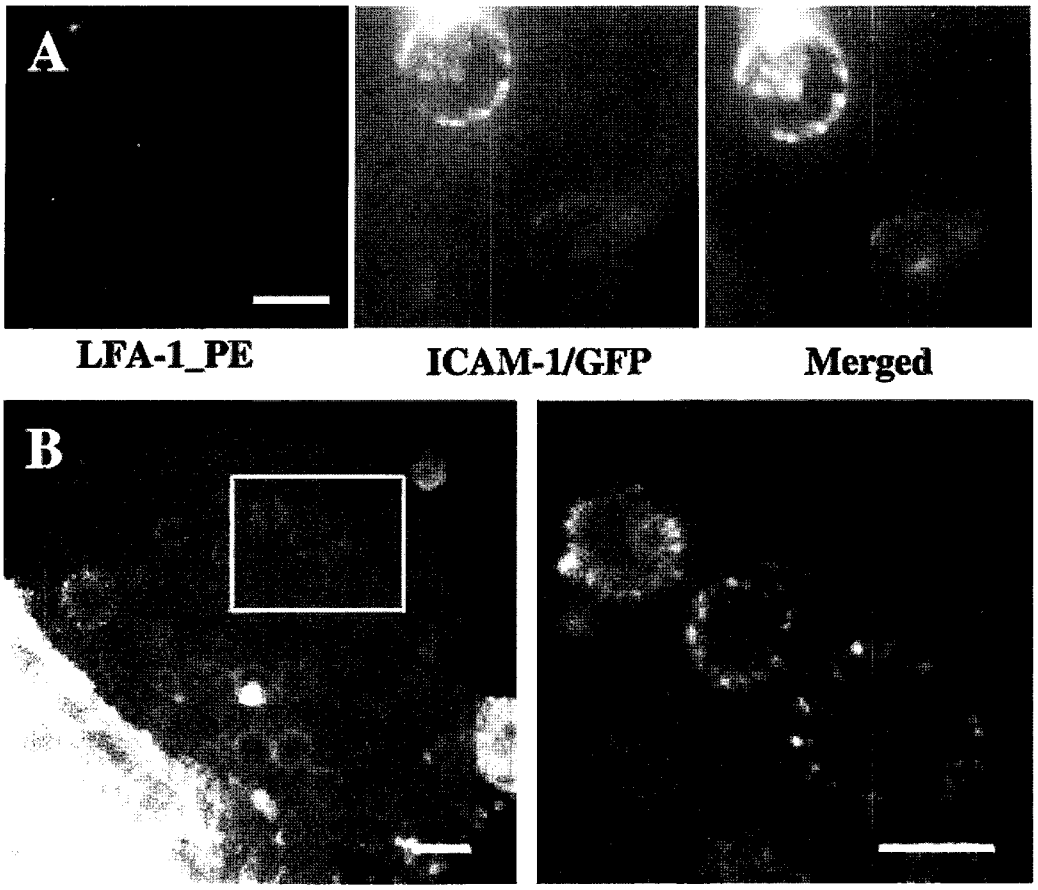

Fig. 2. Evaluation of ICAM-1/GFP clustering on CHO cells. A, Co-localization of LFA-1 and ICAM-1/GFP at the interface between two cells. LFA-1 on K562 cells was labeled with TRITC-conjugated LFA1/2 mAb and then the cells were incubated with IC1_GFP/CHO cells for 20 min. At the end of incubation, cells were washed and the distribution of LFA-1 and ICAM-1 was examined under the fluorescence microscopy. B, Visualization of contact area between IC1_GFP/CHO and LFA-1$^+$ K562 cells. LFA-1$^+$ K562 cells were incubated with IC1_GFP/CHO cell monolayers as described in Fig. 1C and D, redistribution of ICAM-1/GFP was examined under the fluorescence microscopy with different magnifications. Bar = 10 μm.

modified the conventional cell adhesion assay, as described in Materials and Methods, and assessed the ability of IC1_GFP/CHO cells to support adhesion of LFA-1$^+$ K562 cells. As Fig. 1C shows, while the basal binding of LFA-1$^+$ K562 cells to IC1_GFP/CHO was relatively inefficient, incubation with CBR LFA1/2 mAb, a LFA-1 activation antibody, dramatically increased binding, indicating that ICAM-1/GFP is functionally active and the binding is specifically mediated by LFA-1-ICAM-1 interaction. Interestingly, binding of LFA-1$^+$ cells onto the IC1_GFP/CHO cells rapidly induced ICAM-1/GFP clustering at the marginal zone of contact area, as assessed by fluorescence microscopy (Fig. 1D). A functional blocking antibody, RR1/1, could also completely block the cell binding (data not shown).

Clustering of ICAM-1/GFP at the interface between LFA-1$^+$ K562 cells and IC1_GFP/CHO cells

To verify whether the clustering of ICAM-1/GFP is dependent on the mobilization of LFA-1, LFA-1 on K562 cells was labeled with TRITC-conjugated CBR LFA1/2 mAb, incubated with IC1_GFP CHO cells, and examined fluorescence signal under the microscope. As expected, LFA-1 was co-localized with ICAM-1/GFP, indicating that the ICAM-1 clustering is dependent on LFA-1 mobilization after binding (Fig. 2A). Interestingly, the accumulated ICAM-1/GFP at the contact area revealed a special feature in the fluorescence microscopy (Fig. 2B). This unexpected feature was similar to focal adhesion (FA), a specific region within plasma membrane, where actin stress fibers and associated proteins are anchored (Smilenov et al., 1999). To verify whether this FA-like structure is due to focal concentration of ICAM-1/LFA-1 in the cell-cell contact area, we carried out confocal microscopy. Interestingly, the result of the confocal microscopy revealed that although ICAM-1/GFP was accumulated in the cell-cell contact area, there was no suggestive sign of focal adhesion. Instead, ICAM-1/GFP cells projected plasma membrane and encircled LFA-1$^+$ K562 cells. The height of projection was ~10 μm as defined by z-section of confocal microscopy (Fig. 3A). In addition, cross-sectional 2D reconstruction revealed that it does not show

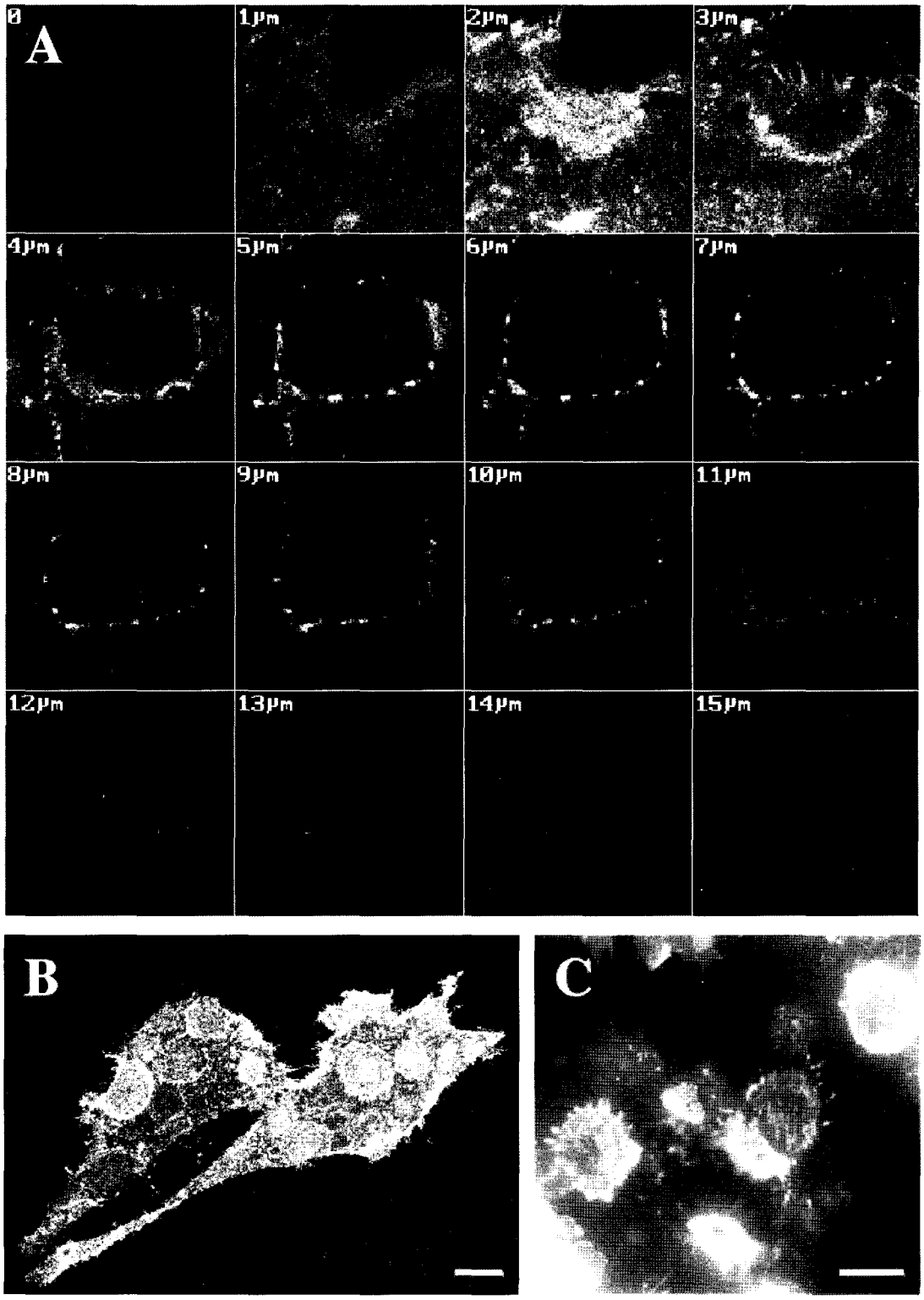

Fig. 3. Characterization of ICAM-1 clustering by confocal or fluorescence microscopy. A, IC1_GFP/CHO cells were incubated with LFA-1^{+} K562 cells in the presence of LFA1/2 mAb. After 20 min of incubation, the cells were washed and fixed. Fluorescence image was acquired by using confocal microscopy with z-axis section to provide representative cross-sectional images. B, Cross-sectional images acquired from A were reconstituted as a two-dimensional image. C, Visualization of ICAM-1 clustering on HUVEC. HUVEC were pretreated with IL-1β for 16 h and then the cells were washed and treated with Cy3-conjugated RR1/1 Fab fragments. After incubation with LFA-1^{+} K562 cells for 20 min, images were visualized under the fluorescence microscopy. Bar = 10 μm.

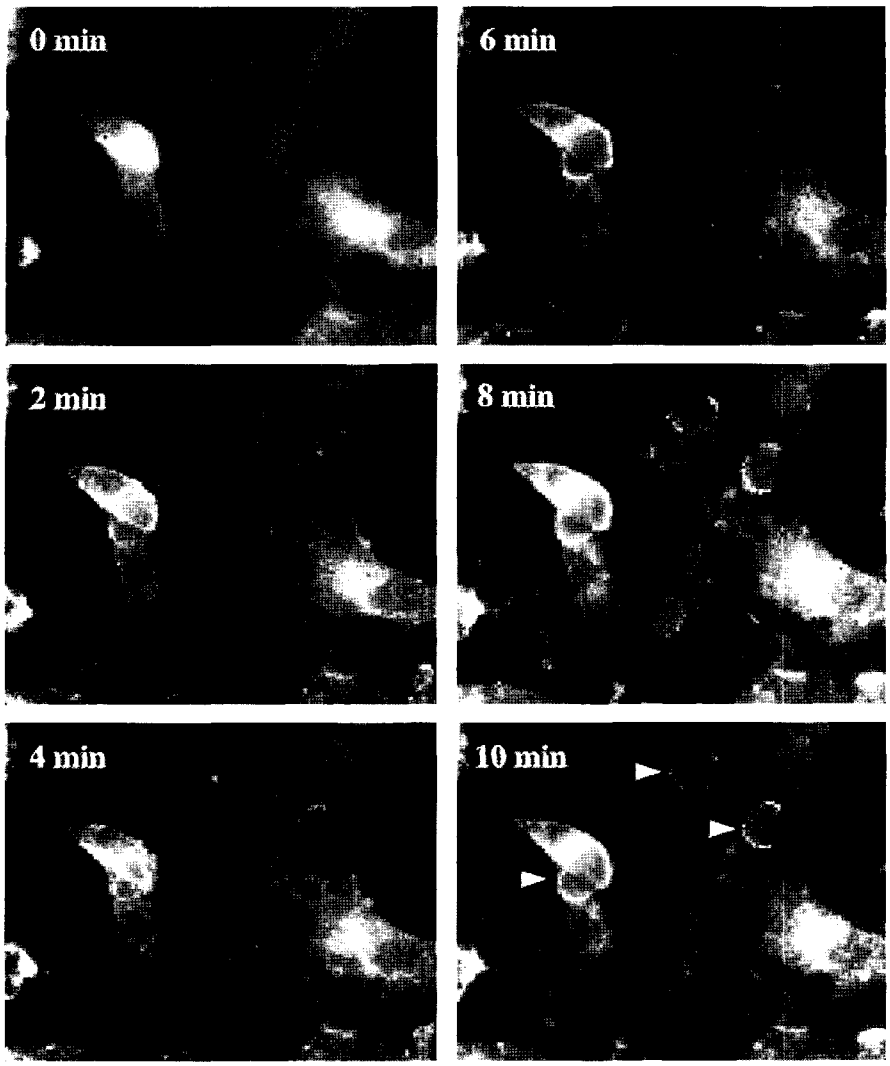

Fig. 4. Kinetics of ICAM-1/GFP accumulation during LFA-1$^+$ K562 cell recognition of IC1_GFP/CHO cells. LFA-1$^+$ K562 cells activated by LFA1/2 mAb were added on the IC1_GFP/CHO cells and the mobilization of ICAM-1/GFP during cell adhesion was monitored by the fluorescence microscopy at various time intervals.

a sign of special accumulation like FA (Fig. 3B). To further understand whether this phenomenon can be also seen during the HUVEC interaction with LFA-1$^+$ cells, cultured HUVEC were activated with IL-1β for 16 h, labeled with Cy3-conjugated RR1/1 Fab frgment, and further incubated with LFA-1$^+$ K562 cells. As shown in Fig. 3C, the pattern of ICAM-1 clustering on HUVEC after interaction with LFA-1$^+$ cells was similar to that of IC1_GFP/CHO cells.

The ICAM-1/LFA-1 adhesion and the following clustering were susceptible to inhibition at low temperature (4℃) in cell-cell binding (data not shown), indicating requirement for membrane fluidity or metabolic energy. Even though the first accumulation was detectable within seconds, ICAM-1/GFP kept accumulating over several minutes before reaching plateau that is maintained for at least several hours (data not shown). The average accumulation time to reach plateau was 10±3 min (Fig. 4).

Both adhesion and clustering of ICAM-1/LFA-1 are independently regulated from actin cytoskeleton and myosin light chain kinase

It has been reported that cross-linking of external domain of endothelial ICAM-1 molecules with antibodies leads to clustering of ICAM-1 molecules and redistribution of endothelial actin cytoskeleton (Wojciak-Stothard et al., 1999). Inhibition of actin stress-fiber with cytochalasin D results in alteration of the cellular localization of ICAM-1 (data not shown). We next evaluated whether cytochalasin D could also affect direct binding of LFA-1 to ICAM-1. Although pretreatment of IC1_GFP/CHO cells with cytochalasin D altered distribution of ICAM-1/GFP and actin on CHO

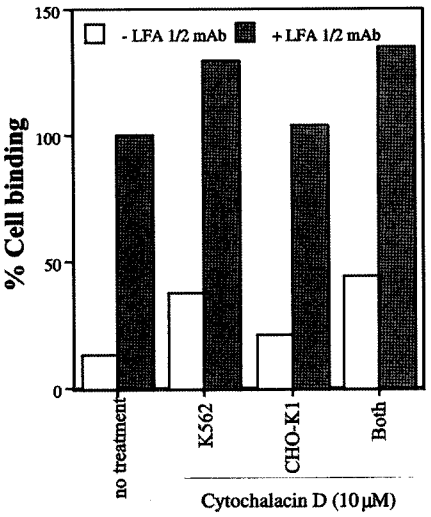

Fig. 5. Effect of cytochalacin D in the binding of LFA-1$^+$ K562 cells on IC1_GFP/CHO cells. LFA-1$^+$ K562 cells and/or IC1_GFP/CHO cells were treated with cytochalasin D for 1 h, and then both cells were further incubated for 20 min at 37℃. Percent binding of LFA-1$^+$ cells to IC1_GFP/CHO cells was calculated as described in *Materials and Methods*. Results are expressed as mean ±SD of triplicate samples.

cells, it did not inhibit the adhesion of LFA-1$^+$ cells to IC1_GFP/CHO cells (Fig. 5). Furthermore, similar clustering event was observed after binding of LFA-1$^+$ cells to IC1_GFP/CHO cells. This result implies that the clustering of ICAM-1 after LFA-1 binding is independent of the signal pathway coupled with actin stress-fiber formation. We also addressed whether the ICAM-1/GFP clustering is linked to the activity of myosin light chain kinase (MLCK), as was suggested for the Rho-mediated assembly of integrins into focal contacts (Burridge and Chrzanowska-Wodnicka, 1996; Chrzanowska-Wodnicka and Burridge, 1996). A MLCK inhibitor, BDM, acts as an inhibitor of muscle myosin ATPase activity (Higuchi and Takemori, 1989). BDM caused a loss of stress fibers in CHO cells, but did not inhibit the ICAM-1/LFA-1 adhesion following the induction of clustering (Fig. 6). These results show that myosin-dependent contractility is not necessary for the ICAM-1 clustering.

Discussion

In vivo, leukocyte adhesion to endothelial cells is a prerequisite for subsequent transmigration across the endothelium into underlying tissues. It has been proposed that ICAM-1 plays a key role in the leukocyte-endothelium adhesion through its recognition of β2 integrin counter-receptors LFA-1 and Mac-1. In this paper we have demonstrated an experimental system that allows one to simultaneously follow morphological changes and distribution of ICAM-1 on live cells in real time. Engineered CHO cell lines stably expressing human ICAM-1/GFP could efficiently support adhesion of LFA-1$^+$ K562 cells, as determined by the use of a functional blocking antibody, RR1/1. The accumulation was detectable within seconds and ICAM-1/GFP kept accumulating until ~10 min. The accumulated ICAM-1/GFP in the contact area revealed unexpected special feature, similar to focal adhesion (FA). However, confocal microscopy revealed that this FA-like structure is due to the projected membrane of ICAM-1/GFP CHO cells encircling LFA-1$^+$ cells. The height of projection was ~10 μm as defined by z-section of confocal microscopy. Clustering of ICAM-1 induced by LFA-1 might not depend on actin cytoskeleton and the signal pathway of stress fiber formation, as it was not prevented by cytochalacin D, an inhibitor of actin polymerization, and BDM, a MLCK inhibitor.

It is well known that ICAM-1 serves as an accessory molecule for T cell activation. T cell activation is mediated by the interaction of T cell antigen receptors (TCRs) with their ligands, major histocompatibility molecule-peptide complexes (MHC-peptide) (Unanue, 1984). Initial views of antigen-specific T cell junctions have revealed formation of a specialized contact, termed the immunological synapse (Monks et al., 1998; Paul and Seder, 1994). The mature immunological synapse is defined by a specific pattern of receptor segregation with a central cluster of TCRs surrounded by a ring of integrin family adhesion molecules (Monks et al., 1998). In this report, we found that interaction of LFA-1$^+$ cells with ICAM-1/GFP CHO cells showed no segregation of ICAM-1 in the contact area between the two cells. Instead, ICAM-1 bearing cells projected membrane and surrounded LFA-1$^+$ cells without any specialized form of contact. This indicates that molecular interaction during transmigration process is quite different from that of T cell binding to APC (Grakoui et al., 1999).

A recent paper demonstrated that adhesion of leukocyte binding to endothelial cells needs actin cytoskeleton and subsequent Rho signaling pathways (Wojciak-Stothard et al., 1999). However, in our system, treatment of ICAM-1/GFP CHO cells with cytochalasin D showed little effect on the ICAM-1/LFA-1 adhesion and clustering. Although cytochalacin D-treated cells showed almost complete disruption of cytoskeleton, it did not affect clustering of leukocyte-binding receptor, ICAM-1, as determined by fluorescence microscopy. Although our result shows discrepancy with the previous study (Wojciak-Stothard et al., 1999), other report strongly supports our finding. For example, cells expressing ICAM-1 without cytoplasmic domain (ICAM-1 Δcyt) mediate adhesion, to a level similar to the one obtained with wild-type ICAM-1, but can no longer support PMN transmigration (Sans et al., 2001). In addition, it has been well established that purified ICAM-1 supports leukocyte binding (Jun et al., 2001). ICAM-1/GPI, which is attached to the cell membrane via a lipid, could also support JY cell binding (Miller et al., 1995). Very interestingly, we found that the engagement of LFA-1 to ICAM-1/GPI could induce

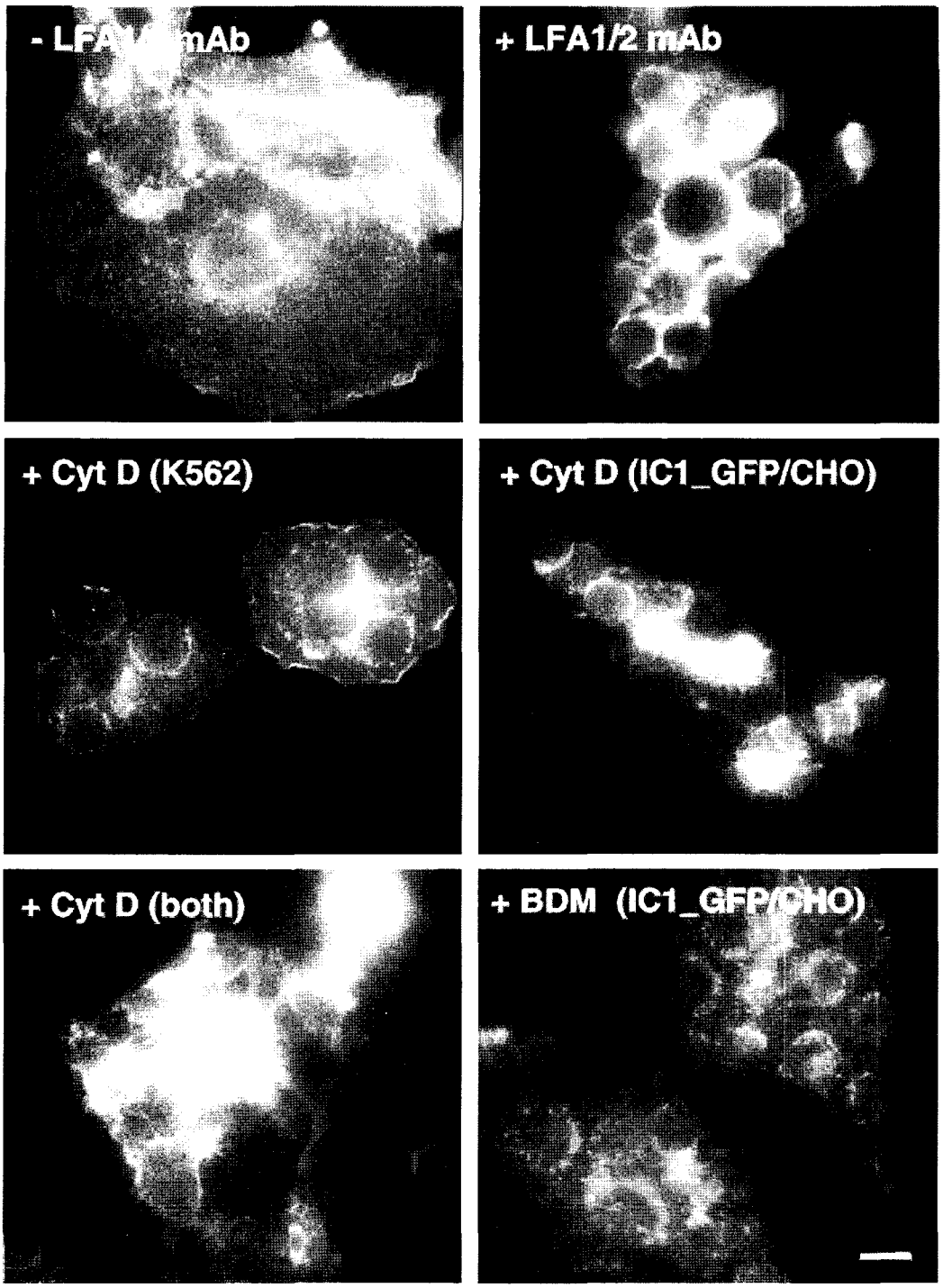

Fig. 6. Effect of cytochalacin D and BDM on ICAM-1 clustering. LFA-1$^+$ K562 cells and/or IC1_GFP/CHO cells were treated with cytochalasin D for 1 h and then the cells were co-incubated in the presence of LFA1/2 mAb for 20 min. The distribution of ICAM-1/GFP was examined under the fluorescence microscope. Bar = 10 μm.

ICAM-1 clustering (data not shown), indicating that intracellular signaling cascade is not necessary for ICAM-1 binding to LFA-1 and subsequent receptor-ligand clustering. Despite strong evidence as described above, however, we cannot rule out possible roles of other receptors such as VCAM-1, PECAM-1, and E-selectin on ICAM-1 clustering after ligand engagement. Because CHO cells originally do not express those receptors, artificial transfection of ICAM-1 cDNA may alter the nature of this molecule and subsequent

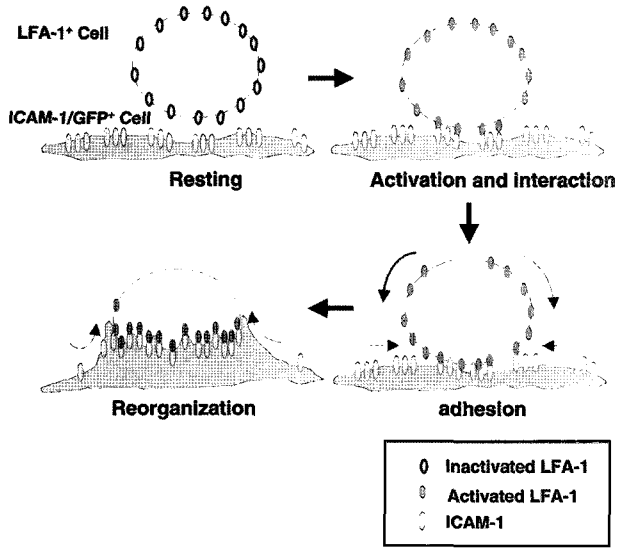

Fig. 7. Schematic model of the events related to LFA-1/ICAM-1 interaction. After LFA-1 activation by several agonists, including activation antibodies, LFA-1$^+$ cells can strongly interact with ICAM-1 and induce clustering of ICAM-1 on CHO cells. This interaction can also induce receptor/ligand reorganization at the interface between two cells.

signaling pathway. On the other hand, recent reports suggest that nitric oxide, a radical produced in mammalian cells from arginine in a reaction catalyzed by NO synthase, is critical in the regulation of leukocyte-endothelial cell interactions (Lefer et al., 1999; Scalia et al., 1999). Since IL-1β can stimulate endothelial cells to express NOS gene, NO may have functions in both expression and function of ICAM-1.

ICAM-1 has been shown to exist as a dimer on the cell surface and such dimerization appears to enhance the binding to LFA-1 (Miller et al., 1995). The dimeric structure may also have an advantage for receptor-ligand interaction and subsequent clustering. Recently we proposed a model of ICAM-1 topomers on the cell surface in which ICAM-1 represents three major morphologies, including ring-like dimer, open dimer, and "W"-tetramer (Jun et al., 2001). In the model, because the extracellular domain of ICAM-1 is flexible enough to form different topomers, interconversion among those topomers may have important consequences for regulating cell adhesion and receptor clustering. Based on our data in this report and previous findings, we propose a model for ICAM-1 clustering after LFA-1 engagement (Fig. 7). Highly activated LFA-1 can bind to ICAM-1 expressed on the surface of endothelial cells. This binding can subsequently induce receptor-ligand complexes. Finally, the plasma membrane of ICAM-1$^+$ cells would be projected to encircle LFA-1$^+$ cells.

Taken together, these results implicate ultra-architecture of ICAM-1 clustering in leukocyte adhesion to endothelial cells via its interaction with LFA-1. Further studies are now in progress to elucidate the molecular mechanism of ICAM-1 signaling in leukocyte transmigration.

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