Overlapping and Selective Roles of Endothelial Intercellular Adhesion Molecule-1 (ICAM-1) and ICAM-2 in Lymphocyte Trafficking

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The integrin LFA-1 interacts with a variety of ligands termed ICAMs. ICAM-1 and ICAM-2 are both expressed on endothelium and serve as counterreceptors during lymphocyte trafficking. In this study, we analyzed their relative contribution to lymphocyte recirculation through lymph nodes and to recruitment into lung and inflamed skin by blocking mAbs against ICAM-1 and ICAM-2 and mice deficient for ICAM-1. The entry of lymphocytes into peripheral and mesenteric lymph nodes was found to be unaffected by the functional deletion of either ICAM-1 or ICAM-2. However, when both pathways were blocked, recirculation through lymph nodes was strongly reduced. Trapping of lymphocytes in the lung after i.v. injection is partly mediated by LFA-1/ICAM interactions; the data presented in this study show an exclusive role of ICAM-1 in LFA-1-dependent lung trapping. Similarly, ICAM-1, but not ICAM-2, was required for the migration of T effector cells into the inflamed skin. These results indicate that ICAM-1 and ICAM-2 have redundant functions in lymphocyte recirculation through lymph nodes, but ICAM-1 is unique in supporting migration into inflamed sites and trapping within the lung.

In vitro data suggested that the two ICAMs have different roles during adhesion and transendothelial migration. Whereas only ICAM-1, together with other endothelial adhesion molecules and selectins, is involved in the adhesion of T cells to endothelial cell...
lines (25–27), both ICAM-1 and ICAM-2 were found to support transendothelial migration of T lymphocytes. However, the functional delimitation of the two endothelial LFA-1 ligands remains controversial. Some studies showed data for a dominant role of ICAM-1 (25), whereas others favored a certain redundancy in the functions of endothelial ICAM-1 and ICAM-2 in T cell transmigration (26–28).

To date, most of our knowledge about the interactions of ICAM-1 and ICAM-2 with LFA-1 is restricted to in vitro data. In vivo studies were partly hampered by the incomplete blocking capacity of the YN-1 anti-ICAM-1 Ab in mice (Hamann et al., unpublished data). We therefore studied the relative contribution of ICAM-1 and ICAM-2 in lymphocyte recirculation using mAbs (KAT-1 against ICAM-1 (29), and 3C4 against ICAM-2) as well as ICAM-1 knockout mice. Two different knockout mice have been described (24, 30). In both mice, no surface expression of ICAM-1 could be observed in various tissues and cell types. Lack of ICAM-1 was shown to result in impaired immune reactions as well as in a limited leukocytosis and lymphocytosis in these mice, indicating that ICAM-1 has a distinct role in extracting cells from the circulation into tissues (24, 30). In contrast, the deletion of ICAM-2 did not result in marked changes in lymphocyte distribution compared with the wild type (31). However, the exact interplay between ICAM-1 and ICAM-2 in lymphocyte recirculation and homing has not been determined to date. In this study, we show that ICAM-1 and ICAM-2 act in a redundant way in the recirculation of lymphocytes through lymph nodes. In contrast, only ICAM-1 was found to be involved in migration of lymphocytes into inflamed tissue and in trapping in the lung.

Materials and Methods

Antibodies

Anti-CD8 (53-6.72) and anti-Mac-1 (M1/70) were obtained from the American Type Culture Collection (Manassas, VA); anti-CD25 (PC6.1) was from M. Nabholz (Epalpingen, Switzerland). Anti-LFA-1 (H35.89.9 (32)) was kindly provided by M. Pierres (Marseille, France); anti-ICAM-1 was from M. Nabholz (Epalinges, Switzerland). Anti-LFA-1 (H35.89.9 (32)) was kindly provided by M. Pierres (Marseille, France); anti-ICAM-1 (Kat-1 (20)) was by K. Kato, H. Yagita, and K. Okomura (Tokyo, Japan); and anti-ICAM-2 (3C4) was generated as described (33). Purification of mAb was performed by affinity chromatography, as previously described (4), or precipitated by ammonium sulfate from serum-free supernatants. Fab were prepared by cleavage with papain and purified, as described (8). Rabbit anti-rat IgG was obtained from DAKO (Hamburg, Germany).

Animals and induction of skin inflammation

Female specific pathogen free reared C57BL/6 and BALB/c mice (8–16 wk of age) were obtained from Bundesinstitut für Risikobewertung (Berlin, Germany) or Charles River (Sulzfeld, Germany). Mice deficient in the ICAM-1 gene were generated as described (24). Homozygous mutant mice were negative for surface-expressed ICAM-1 on leukocytes and endothelial cells. Heterozygous littersmates, previously shown to display normal functions of ICAM-1 (24), were used as controls in some experiments. A cutaneous delayed-type hypersensitivity (DTH) reaction was induced in BALB/c mice, as described earlier (34). In short, mice were skin painted with 0.5% 2,4-dinitrofluorobenzene (DNFB) in acetone-olive oil (4:1) twice (days −21 and −20) and rechallenged with 0.5% DNFB on one flank or one ear pinna 1 day before injection of cells. In the ears, between 0.2 and 0.5% of labeled effector cells are recruited to the inflamed site within 3 h as compared with 1–2% to a larger skin area on the flank (34); however, the lower background of the ears made this target site more reliable.

Cell preparation

Lymphocytes were prepared from pooled pLN and mesenteric lymph nodes (mLN) of wild-type mice.

For the isolation of CD4+ T cells, lymphocytes were incubated at 4°C with a mAb mixture containing anti-CD8, anti-CD25, and anti-Mac-1. After incubation, cells were washed and depleted from non-CD4+ T cells by two subsequent panning steps on petri dishes coated with rabbit anti-rat Ig (100 μg/ml, cross-reacting with mouse IgG) at room temperature. The average purity of CD4+ T cells after panning was >96%, as determined by FACS analysis.

To generate Th1 cells, CD4+ T cells were cultured on six-well tissue culture plates coated with anti-CD3 (1 μg/ml) and anti-CD28 (3 μg/ml) in the presence of murine rIFN-γ (mIFN-γ) (20 ng/ml), mIL-12 (5 ng/ml), and anti-IL-4 (1–5 μg/ml). After 2 days, the cells were removed from Ab-coated plates and cultured for additional 4 days before use. To verify the polarization into Th1, the intracellular expression of IFN-γ was determined on day 5 (Fig. 1).

In vivo homing assays

The analysis of lymphocyte migration in vivo was conducted as described (8). In short, freshly isolated lymphocytes or Th1 cells at 1 × 107 cells/ml were incubated with 750 kBq/ml sodium 51Cr chromate in RPMI 1640 containing 10% FCS at 37°C. For the analysis of lymphoblast homing, freshly isolated lymphocytes were incubated with 51Cr-labeled wild-type lymphocytes were injected with or without added Fab of mAb (300 μg per mouse) into the tail vein. Mice were sacrificed after 1 h, and the distribution of radioactive activity in various organs and the remaining body was measured. In case of Th1 cell homing into inflamed DTH skin, mice were sacrificed after 3 h. Approximately 80–90% of injected activity was recovered at these time points in the total animal. pLN consisted of a pool of the superficial inguinal nodes, the brachial and axillary nodes, and the superficial cervical nodes. All Peyer’s patches (PP) were collected and counted separately from the remaining gut. PBL values were computed for a volume of 1.5 ml. Each sample was counted to 3% statistical error. The mean values and their SD were determined from three to six mice. For statistical analysis, the Mann-Whitney test was used.

In control experiments, various surface-reactive Fab were tested as isotype controls and showed no influence on the migration of lymphocytes into inflamed and noninflamed tissues (data not shown).

Results

Recirculation of lymphocytes through pLN, mLN, and PP

LFA-1 has been shown to be involved in the recirculation of lymphocytes, especially through pLN. To identify the involved ICAM ligands, radiolabeled wild-type lymphocytes were injected i.v. into C57BL/6 mice with added Fab of mAb against ICAM-1, ICAM-2, or both ICAMs (Fig. 2A). Entry into pLN, mLN, and PP was not influenced by the addition of mAb against ICAM-1 alone. Surprisingly, also blockade of ICAM-2, even by mAb doses as high as 500 μg of Fab per mouse, was without effect. Thus, neither ICAM-1 nor ICAM-2 alone is absolutely required for lymphocyte entry into the lymph nodes. However, after simultaneous blockade of both adhesion molecules, ICAM-1 and ICAM-2, a drastically decreased migration, by almost 80%, of lymphocytes into pLN was observed, similar to effects found upon blocking or deletion of LFA-1 (4, 5). Additionally, the entry into mLN and PP was diminished by ~40% (p < 0.05 in all three organs).

![Figure 1](Image)

**FIGURE 1.** Cytokine production and selectin ligand expression in day 5 Th1 cultures. Naive CD4+ cells stimulated with anti-CD3 and anti-CD28 in presence of IL-12 and IFN-γ, as described in Materials and Methods, were restimulated with PMA/ionomycin in the presence of brefeldin A, fixed, permeabilized, and stained for intracellular IFN-γ, as described (34). Expression of ligands for inflammatory selectins was analyzed by staining with P-selectin Ig, as described (34). Few (0–8%) cells in such cultures produced IL-4 (data not shown).
The findings were confirmed by injecting radiolabeled wild-type lymphocytes into mice deficient for ICAM-1 and the additional blockade of ICAM-2 with mAb (Fig. 2B). Entry into pLN was only slightly diminished in ICAM-1-deficient compared with the normal mice (Fig. 2B, p < 0.05). No effect at all was detectable in the mLN and the PP (Fig. 2B), and the additional blockade of LFA-1 in ICAM-1-deficient mice showed only weak effects in mLN and PP (data not shown). However, mAb against LFA-1 blocked the immigration into pLN in ICAM-1-deficient mice by 50–80% (data not shown), as was described previously for ICAM-1−/− mice (4, 5, 35). These findings suggest that the alternative ligand for LFA-1 available on endothelium, ICAM-2, is responsible for lymphocyte trafficking through high endothelial venules in pLN. However, when both pathways were blocked by combining ICAM-1-deficient mice with the additional application of anti-ICAM-2 mAb, a strong reduction (60%) of lymphocyte entry into pLN was observed, approaching the lower entry rates obtained in the presence of anti-LFA-1 Abs. The same results were obtained by injecting CD4+ T cells instead of lymphocytes (data not shown). Although a contribution of the LFA-1/ICAM pathway for immigration into mLN and PP has previously been demonstrated using Abs or knockout animals (4, 5), the observed effects are smaller and therefore not detected with certainty in all experiments. We also cannot exclude that a shift in relative contribution of adhesion pathways for these organs occurs in the knockout model. These data indicate that both ICAM-1 and ICAM-2 are involved in the extravasation of lymphocytes into pLN and can largely substitute for each other.

Trapping of lymphocytes in the lung

Circulating lymphocytes are retarded for a certain time within the lung (11, 36). It has been shown that, at least in part, an LFA-1-dependent adhesion mechanism accounts for this event (4). Deficiency in the ICAM-1 pathway alone already resulted in a decreased trapping (30–40% reduction) of lymphocytes in the lung (Fig. 2A, p < 0.05), in contrast to the results for lymphocyte migration into pLN. This reduction was not further reduced by coinjection of anti-LFA-1 (data not shown). Blocking of ICAM-2 alone did not result in reduced lymphocyte numbers in the lung (Fig. 1A), and the blockade of ICAM-2 in animals lacking ICAM-1 did not further reduce the lung localization (Fig. 2B). The same results were obtained by injecting CD4+ T cells (data not shown).

Additionally, we investigated the LFA-1-dependent trapping of lymph node blasts in the lung, because these cells show a strong preference for this organ (37). The blasts are similar in this respect, because deficiency for ICAM-1 on the lung endothelium leads to nearly 30% decreased trapping of these cells, which cannot be strengthened by the supplementary injection of anti-LFA-1 mAb (Fig. 3A). This is confirmed by the finding that the blockade of ICAM-2 did not change the trapping of blasts in the lung (Fig. 3B), suggesting that ICAM-1 serves as the only counterreceptor in LFA-1-dependent trapping of resting and activated lymphocytes in the lung.

FIGURE 2. Role of ICAM-1 and ICAM-2 in the recirculation of lymphocytes. Lymphocytes were isolated from pooled pLN and mLN, labeled with 31Cr, and injected into the tail vein. Radioactivity was counted in the organs 1 h after injection. A. Migration pattern of lymphocytes in C57BL/6 mice in the absence or presence of anti-ICAM-1 (∇), anti-ICAM-2 (○), or anti-ICAM-1 and ICAM-2 Fab together (■) (300 μg each/mouse). B. Migration pattern of wild-type lymphocytes injected into heterozygous ICAM-1 mice as control (□), into ICAM-1-deficient mice (○), or into ICAM-1-deficient mice together with 100 μg anti ICAM-2 Fab (■). Mean values and SDs were from n = 3–4 animals. Shown is one representative of 2–4 independent experiments. Injection of various Fab isotype controls had no influence on migration patterns. The different absolute levels of immigration of control cells in A and B reflect usual physiological variability between experiments.

FIGURE 3. Role of ICAM-1 and ICAM-2 in the localization of lymphoblasts in the lung. Lymphocytes from C57BL/6 mice were isolated from pooled pLN and mLN. Cells in the S phase were labeled with 125I-labeled IUdR and injected into the tail vein of ICAM-1-deficient mice (Fig. 3A, lung B, ICAM-1−/−) together with 300 μg anti LFA-1 Fab. B. Role of ICAM-2 for wild-type blast; trapping in the lung was investigated in C57BL/6 mice without injected mAb (□), with anti-ICAM-2 (○), or with anti-LFA-1 Fab (■). Mean and SDs were from n = 3–6 animals. Shown is one representative of two independent experiments.
Migration of Th1 CD4+ T cells into the inflamed skin

To analyze the LFA-1-dependent entry of effector T cells into inflamed tissue, we studied the migration properties of Th1 effector T cells in a cutaneous DTH reaction. After priming with DNFB, challenge of the skin leads to a recruitment of a small fraction of i.v. injected radiolabeled effector cells (34). A striking difference in the mechanism of immigration into inflamed tissue was found compared with the entry into lymphoid tissues. The blockade of ICAM-1 on inflamed skin endothelium leads to a 75% decreased accumulation of Th1 cells in the tissue, which could not be significantly enhanced by the additional blockade of ICAM-2. The single blockade of ICAM-2 resulted only in a minor, not significant reduction of Th1 cell immigration (Fig. 4A). In the same mice, entry of the Th1 cells into unaffected pLN and into draining lymph nodes is controlled by ICAM-1 and ICAM-2 together, because immigration is inhibited only by blockade of both ICAMs together (Fig. 4B). Thus, ICAM-1 is the major endothelial guard for LFA-1-dependent entry into an inflamed tissue, but even in the lymph nodes draining the inflamed area, ICAM-1 can be substituted by ICAM-2.

Discussion

The existence of different ligands for one and the same receptor, such as the ICAMs for LFA-1, is puzzling, as it is not obvious whether evolution created redundant mechanisms or whether specific roles can be assigned to the different molecules. In the case of ICAM-1, a variety of studies suggested that this molecule has a specific role for the inflammation-induced recruitment of lymphocytes into tissues. ICAM-1, but not ICAM-2, becomes up-regulated on most types of endothelium (and other cell types) by proinflammatory cytokines such as IL-1 or TNF-α (12, 38); blocking or deletion of ICAM-1 reduces the infiltration of lymphocytes into inflamed tissues (24, 39); and several studies provided evidence for an anti-inflammatory effect of anti ICAM-1 mAb in inflammatory diseases (40, 41). However, in some types of endothelium, especially the high endothelial venules of lymph nodes, ICAM-1 is expressed constitutively, indicating a role for lymphocyte trafficking under physiological conditions as well.

The role of ICAM-2, the second ICAM ligand for LFA-1 expressed on endothelium, remained less obvious. Its constitutive expression on a large variety of endothelia and the higher expression levels compared with ICAM-1 (at least as far as can be judged from immunohistochemistry) (12) would suggest a general role in physiological mechanisms of leukocyte extravasation. However, neither leukocytes nor lymphocytes extravasate in significant numbers to nonlymphoid tissues in the absence of inflammation, except in lung or liver, and no obvious defects in lymphocyte trafficking were observed in ICAM-2-deficient animals (31).

The findings of this study clarify the functional division of labor between ICAM-1 and ICAM-2 with respect to lymphocyte trafficking. First, both ICAM-1 and ICAM-2 have an important, overlapping role in the physiological mechanism of lymphocyte recirculation through lymph nodes and PP. ICAM-1 and ICAM-2 are able to substitute for each other to a large degree, as blocking of only one molecule has little effect on lymphocyte entry into these lymphoid organs. This explains why in ICAM-2-deficient animals lymphocyte homing to lymph nodes was found to be unimpaired (31). Second, a role for ICAM-2 in lymphocyte localization within other organs, in presence or absence of ICAM-1, could not be detected, despite its ubiquitous expression on various endothelia of the body. This applies also for LFA-1-dependent lymphocyte trapping within the lung. Third, T effector cell recruitment into an inflamed tissue such as skin is only mediated by ICAM-1.

LFA-1 and ICAMs have no role in the low rate of entry of lymphocytes into the normal skin, despite presence of ICAM-2 on endothelium (12). In contrast, increased recruitment into a cutaneous DTH reaction site is largely dependent on LFA-1 (data not shown) and on ICAM-1, whereas blocking of ICAM-2 has almost no effect. Strong up-regulation of ICAM-1 upon inflammatory stimuli is known for a variety of inflamed tissues (19, 20), in contrast to expression of ICAM-2 being refractory to inflammatory mediators (12). In addition, various studies reported a protective influence of ICAM-1 deficiency in some diseases (42, 43).

Circulating lymphocytes become transiently trapped within the lung. This applies to normal lymphocytes, but even more to activated/memory lymphocytes, and is partially mediated by LFA-1-dependent adhesion (4, 37, 44). As most of the cells reappear within the first hour or, in case of activated/memory cells, after several hours within the circulation (37), it is probable that these cells are rather marginalized within the vasculature than truly extravasated. Neither to which type of cells the circulating lymphocytes adhere via LFA-1 nor to which ligands LFA-1 binds in this case is known.

Of special interest was, therefore, the finding that ICAM-1, but not ICAM-2, is involved in lymphocyte localization within the lung. From several studies, an enhanced expression of adhesion molecules including ICAM-1 in the pulmonary vasculature during inflammation is well documented (41, 45, 46). Additionally, it has been shown that blockade or deficiency of ICAM-1 contributes to prevent disease in some inflammatory lung models (41, 47). Nevertheless, in some other forms of lung inflammation, it appears that ICAM-1 is not up-regulated or even not required for adhesion (48, 49). However, in the normal lung, ICAM-1 is expressed constitutively at high levels throughout the endothelial surfaces (46), and the expression seems to be already higher compared with other tissues (50). Our findings apparently contradict a recent study using intravital microscopy in perfused rat lungs, in which the investigators were unable to detect an effect of blocking ICAM-1 on leukocyte entrapment in the lung (51). Apart from the different
populations studied, this discrepancy might relate to inherent drawbacks of the intravital method or to insufficient blocking activity of the used mAb.

The failure of ICAM-2 to support extravasation into nonlymphoid organs, such as lung and liver, where a significant trapping or even transmigration of lymphocytes occurs regularly (36), is surprising in the light of its widespread expression. In vitro data using recombinant proteins show a lower affinity of ICAM-2 to LFA-1 (12); it is conceivable that this counteracts the high expression levels of ICAM-2. In consequence, binding of cells to endothelia expressing only ICAM-2 might require stronger activation of LFA-1, e.g., by chemokines present only in lymph nodes. However, blocking effects of ICAM-2 mAb were also lacking when lymphoblasts were studied. Blasts were previously shown to express higher levels and functionally activated LFA-1 (52, 53). In frozen section assays of chronically inflamed human airway endothelium, an influence of ICAM-2 in the binding of T cells has previously been detected (54), whereas in the inflamed lungs of ICAM-2-deficient mice a compromised accumulation of eosinophils, but not of lymphocytes, was demonstrated (31). Similarly, numbers of lymphocytes in the bronchoalveolar lavage of untreated ICAM-2-deficient mice were normal (31), indicating that ICAM-2 is dispensable for transmigration of lymphocytes in this organ.

As found previously, blocking of the ICAM/LFA-1 pathway as well as of other receptors relevant for the recirculation of naive lymphocytes results in increased cell numbers in the spleen (8, 53, 55). This confirms earlier findings that the investigated molecules are not required for lymphocyte recirculation through the spleen, and simultaneously suggests a role of the spleen as a buffer for elevated cell numbers in the circulation, as hypothesized previously (5).

In accordance with earlier studies blocking or deleting LFA-1 (4, 5, 35), the blocking of both ICAM-1 and ICAM-2 does not completely prevent lymphocyte immigration into pLN and has only small, not always detectable effects on the entry into mLN and PP. Data in LFA-1-deficient mice reveal that, in addition to the redundancy between the ICAMs, an overlap in the function of β2 and αL integrins also exists. Hereby, a distinctive role of VCAM-1 in pLN, and VCAM-1 and mucosal addressin cell adhesion molecule-1 in mLN and PP, respectively, can be ascribed in addition to the ICAM/LFA-1 adhesion pathway (5).

In this conclusion, this study identifies an overlapping role of ICAM-1 and ICAM-2 in lymphocyte recirculation through lymph nodes, excludes unique functions of ICAM-2 in physiological homing mechanisms, and shows that exclusively ICAM-1 is involved in migration into inflamed skin and in trapping within the lung.

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References


