Identification of an Active Sequence within the First Immunoglobulin Domain of Intercellular Cell Adhesion Molecule-1 (ICAM-1) That Interacts with Fibrinogen*

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Monocytic cells bind fibrinogen (fg) through integrin α5β2. fg-bound monocytic cells demonstrate an enhanced adhesion to endothelial cells, which is dependent on intercellular adhesion molecule-1 (ICAM-1). Our studies differentiate fg interactions with stimulated and resting endothelial cells, which are ICAM-1 dependent and independent, respectively. This report documents a direct interaction between fg and intact ICAM-1 and with a two-Ig domain form of ICAM-1. A small region within the first Ig domain of ICAM-1, ICAM-1(8–21) (KVILPRGGSVLVTC), was identified to interact with fg in a specific and selective manner. ICAM-1(8–21) bound to plasmin-derived fg fragments X, D100, and D80 but not to fragment E. Consistent with this finding, fg γ-chain peptide, fg-γ117–133, blocked fg interaction with ICAM-1(8–21). ICAM-1(8–21) peptide and antibodies directed against ICAM-1(8–21) also blocked the adhesion and binding of ICAM-1-bearing Raji cells with fg. ICAM-1(8–21) and fg-γ117–133 are likely to be one of the contact pairs mediating fg-ICAM-1 interactions.

While fibrinogen (fg) is a plasma protein and intracellular adhesion molecule 1 (ICAM-1) is primarily a cell surface protein, both play central roles in cell-cell interactions (1, 2). fg, a dimeric 340-kDa molecule, circulates in blood at 2–3 mg/ml. It protein, both play central roles in cell-cell interactions (1, 2). fg, a dimeric 340-kDa molecule, circulates in blood at 2–3 mg/ml. It protein, both play central roles in cell-cell interactions (1, 2).

EXPERIMENTAL PROCEDURES

Reagents and Antibodies—TNF-α and interleukin-1 were purchased from Genzyme (Boston, MA). Na235Cl was from Amersham Life Sciences (Arlington Heights, IL), and sodium [51Cr]chromate was purchased from Dupont/New England Nuclear (Wilmington, DE). Anti-VCAM-1 (E1/6) and anti-ICAM-1 monoclonal antibodies (mAbs) (LB-2) were purchased from Becton Dickinson (San Jose, CA). Additional anti-ICAM-1 mAbs, QE2 (20) and WEHI-CAM-1 (21), were generously provided by R. Faulk, Adelaide, Australia, and A. W. Boyd, Melbourne, Australia, respectively. Anti-α5β2 mAb, LM 609 (22), was a gift from D. Cheresh (Research Institute of Scripps Clinic, La Jolla, CA). 7E3 mAb was provided by B. Coller, Mt. Sinai Hospital, New York, NY. Polyclonal anti-ICAM-1 antisera (B803) was produced in the laboratory by...
immunizing rabbits with recombinant ICAM-1 expressed in E. coli (23). Antipeptide antibodies were generated by immunizing rabbits with peptides coupled to keyhole limpet hemocyanin. Antisera were tested for specificity in an enzyme-linked immunosorbent assay format (24).

Cells—EC were harvested from human umbilical cords (25). EC were plated on tissue culture-treated polystyrene (Costar Corp., Cambridge, MA) coated with 1.0 μg/cm² human fibronectin (Boehringer Mannheim, Indianapolis, IN) and grown in Dulbecco’s modified Eagle’s medium F-12 (BioWhittaker, Walkersville, MD) containing 15% fetal calf serum and 180 μg/ml EC growth supplements (Clonetics, San Diego, CA). Cells were initially grown in T75 culture flasks until confluent and 3100 m mole/m² human fibronectin (Boehringer Mannheim, Indianapolis, IN) and grown in Dulbecco’s modified Eagle’s medium F-12 (BioWhittaker, Walkersville, MD) containing 15% fetal calf serum and 180 μg/ml EC growth supplements (Clonetics, San Diego, CA).

Cells were incubated with anti-αβ₃, anti-ICAM-1 (QE2) mAbs, or with a control anti-αmβ₁, mAbs (PMI-1). The disclosing antibody was fluorescein isothiocyanate-conjugated anti-mouse IgG.

Fig. 1. FACS analysis of resting and TNF-α-stimulated EC and Raji cells. Cells were incubated with anti-αβ₃, (LM 609), anti-ICAM-1 (QE2) mAbs, or with a control anti-αmβ₁ mAbs (PMI-1). The disclosing antibody was fluorescein isothiocyanate-conjugated anti-mouse IgG.

Fibrinogen Interactions with ICAM-1

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Fibrinogen and Fibrinogen Fragments—Fg was purified from fresh human plasma by cryoethanol precipitation (30). fg was radioiodinated using Iodo-Beads (Pierce) and dialyzed against 0.3 M NaCl (31, 32). The specific activity of radioalabeled fg was 0.45–0.52 mCi/mg. Plasmin digestion of fg was performed to obtain fragments X, D100, D80, and E (33). Fragments were purified by ion exchange using a 0–200 m NaCl gradient. The molecular masses of fragments X, D100, D80, and E were 245, 100, 80, and 45 kDa, respectively, as estimated from their mobilities relative to protein standards on nonreducing 5–15% gradient SDS-polyacrylamide gel electrophoresis (33). The fragments were dialyzed against phosphate-buffered saline and radioiodinated. The specific activities of fragments X, D100, and D80 were 0.33, 1.52, and 1.4 mCi/mg, respectively.

Fg Binding to ICAM-1 Peptides—Peptides (100 μl) at 0.5 mM in TBS were coated onto Falcon 3911 microtiter plates (Becton Dickinson Labware, Oxnard, CA) for 16 h at 4°C. The peptide solution was removed, and the plates were washed and blocked with 1% bovine serum albumin (BSA) 125I-labeled fg (20 nM) in TBS, containing 1.0 mM CaCl₂, was applied to the wells for 4 h at 3°C. Plates were washed three times with TBS, and the individual wells were counted in a gamma counter (Iso-Data 20/20, San Marcos, CA).

Ligand Blotting—Detergent lysates of SF9 cells transfected with viral vector encoding full-length five-domain and truncated two-domain forms of ICAM-1 were separated by SDS-polyacrylamide gel electrophoresis (34) and electrophoretically transferred to polyvinylidene difluoride Immobilon membranes (Millipore, Bedford, MA) using 10 mM CAPS in 10% methanol at pH 11. The membranes were probed with 125I-fg (20 nM) in TBS containing 0.1% BSA and 1 mM MgCl₂, washed once with TBS containing 0.05% Tween 20 and four times with TBS alone, and then dried and autoradiographed using X-OMat films (Eastman Kodak Company).

Adhesion Assays—Adhesion of EC or Raji cells to immobilized fg was performed as described previously (27). Briefly, cells were washed three times gently with Hank’s balanced salt solution (HBSS) without divalent ions and labeled with 0.5 μl of 31Cr (1 mCi/ml) at 22°C for 30 min. The radiolabeled cells were washed in HBSS, and the final cell pellet was suspended in HBSS containing 0.1% BSA and 2 mM CaCl₂ to give 7.5 × 10⁶ cells/ml. Cells were allowed to adhere to fg-coated wells (1.0 μg/well) for 30 min at 37°C. The nonadherent cells were aspirated, and the adherent population was lysed in a solution containing 2% SDS and 0.2 mM NaOH for 30 min, transferred to vials containing 3 ml of BioSafe scintillation liquid (Research Products International, IL), and counted in a beta counter (LS 3801, Beckman Instruments, Fullerton, CA).
**Fibrinogen Interactions with ICAM-1**

**Fibrinogen Binding to Raji Cells**—Fibrinogen binding was performed as described previously (31, 35). Briefly, cells were washed thr. 3 times in HBSS, \(^{125}\)I-fg (300 nM) was allowed to bind \(5 \times 10^7\) Raji cells in a total volume of 200 µl in HBSS containing 0.1% BSA for 30 min at 22°C. Following incubation, the cell-bound radioactivity was separated from unbound material by passing through a cushion of 20% sucrose by centrifugation at 16,000 rpm for 2.5 min in an Eppendorf centrifuge. The cell pellet was counted on a gamma counter, and molecules of fg per cell was calculated as described (31, 35).

**Fluorescence-activated Cell Sorting (FACS)**—Resting and TNF-α-stimulated EC were isolated from culture flasks by brief trypsin treatment and washed twice in Dulbecco’s phosphate-buffered saline. Cells were resuspended in a staining medium of HBSS containing 20 mM CaCl\(_2\), 20 mM MgCl\(_2\), 10 mM HEPES (pH 7.4), and 0.1% BSA and incubated at 4°C for 30 min with 5.0 µg/ml of control mouse IgG, anti-α\(_v\)β\(_3\) mAb LM 609, anti-ICAM-1 mAbs QE2, or LB-2. Cells were centrifuged through a cushion of fetal calf serum and resuspended in staining medium containing 50 µg/ml fluorescein isothiocyanate-conjugated goat anti-mouse IgG antibodies (Zymed Laboratories, South San Francisco, CA). Cells were incubated for 30 min at 4°C, then centrifuged, and resuspended in staining medium. Cell-bound antibodies were detected by FACSscan using the LYSIS program (Becton Dickinson).

**Molecular Modeling of the First Ig Domain of ICAM-1**—Software InsightII (Biosym Technologies Inc., San Diego, CA) and Homology were used to carry out the modeling. The first Ig domain of VCAM-1 was taken as a template to construct the backbone conformation of ICAM-1. The structure of VCAM-1 was taken from the Protein Data Bank (36). Sequence alignment of ICAM-1 and VCAM-1 was performed to align the two molecules (37). The conformation of the ICAM-1 first-Ig domain backbone was determined in two steps using Homology. Initially, the structurally conserved regions were built. Because ICAM-1 is thought to have the same secondary structures as VCAM-1, we took the strands and the integrin-binding motif as structurally conserved regions. The conformation of structurally conserved regions were determined by copying the coordinates of the corresponding backbone atoms in VCAM-1. The other parts of the molecule were treated as loops. The conformations of these loops were determined by searching the protein conformation data base in the Biosym software. A conformation-generating procedure was used that fit the loops into the structurally conserved regions. The best ten conformations for each loop were displayed and selected manually to find the most suitable ones. The side chains were adjusted to remove bumps to give a preliminary model. Energy minimization was carried out to refine this structure. The model was subjected to 200-steps steepest decent optimization. The final structure was reached by using the conjugate gradient method until the maximum deviations were <0.1 kcal/mol Å.

**RESULTS**

**ICAM-1-Fibrinogen Interactions in Endothelial Cell Adhesion**—Previous studies have yielded conflicting results regarding the roles of the α\(_v\)β\(_3\) integrin and ICAM-1 in mediating fg binding with EC (1, 38). The described interactions have ranged from being entirely blocked by RGD peptides and α\(_v\)β\(_3\) mAbs, characteristics of α\(_v\)β\(_3\)-mediated interactions, to being entirely insensitive to these reagents (7, 39, 40). We hypothesized that these differences might reflect the extent of ICAM-1 expression by EC. Accordingly, the adhesion of EC, either stimulated with TNF-α (10 ng/ml) to up-regulate ICAM-1 expression or in a basal state, to fg was compared. FACS analysis verified that ICAM-1 expression was low on the nonstimulated EC and considerably higher on TNF-α-stimulated EC, (Fig. 1) as previously reported (2). The mean fluorescence intensity with an ICAM-1 mAb was 35.6 for unstimulated EC and 425.5 for TNF-α-stimulated EC. In contrast, α\(_v\)β\(_3\) expression was unchanged by TNF-α stimulation. The mean fluorescence intensities with an α\(_v\)β\(_3\) specific mAb (LM 609) were 43.7 and 48.7 for unstimulated and stimulated EC, respectively.

In adhesion assays, the proportion of TNF-α-stimulated EC adherent to fg was consistently 20–30% greater than that observed with nonstimulated EC. The α\(_v\)β\(_3\) RGD ligand peptide, GRGDSP, inhibited the adhesion of both stimulated and nonstimulated EC; however, the profile of inhibition was significantly different (Fig. 2). At 12.5 µM RGD, the inhibition observed was 70% for nonstimulated EC (Fig. 2A) but only 32% for TNF-α-stimulated EC (Fig. 2B), yet at similar concentrations the control GRGESP peptide had a negligible effect in each case. Similar differences in the inhibition profiles were noted with 7E3, a mAb that blocks α\(_v\)β\(_3\)-mediated function (41). At 2 µg/ml, 7E3 inhibited the adhesion of nonstimulated EC to fg by 71%, whereas the adhesion of stimulated EC to fg was only 46% inhibited.

The differences in these inhibition profiles suggest that a component of stimulated EC adhesion to fg may be α\(_v\)β\(_3\) independent. The contribution of ICAM-1 to this α\(_v\)β\(_3\)-independent adhesion was verified using an anti-ICAM-1 mAb (LB-2) at 20 µg/ml in adhesion assays. The ICAM-1 mAb decreased the adhesion of stimulated EC to fg by 53%, whereas a control mAb had no effect (Fig. 2B). In contrast, the anti-ICAM-1 mAb had
Identification of the Fibrinogen-interactive Sequence in ICAM-1—On the basis of the above-noted reactions of fg with the first two Ig motifs, we focused on several overlapping peptides within the first two Ig-like domains of ICAM-1 (42) and peptides within VCAM-1, ICAM-3, and integrins as additional controls (Table I). Peptides were immobilized to microtiter plastic wells, and 125I-fg (20 nM) was added to each well in the presence and absence of the reducing agent was identical; in each case, ICAM-1-(8–21) eluted as a single major peak at 0–100% acetonitrile gradient. Furthermore, ICAM-1-(8–20), lacking the COOH-terminal cysteine, also exhibited fg-binding activity, albeit to a lesser extent than ICAM-1-(8–21) (Table I). These results indicate that the fg-binding activity of ICAM-1-(8–21) was not due to dimerization and that the cysteine residue was itself important in the formation of a stable peptide conformation able to interact with fg.

Specificity of fg-ICAM-1-(8–21) Interaction—The binding of fg to ICAM-1-(8–21) was highly specific. First, under conditions in which 125I-fg bound avidly to ICAM-1-(8–21), other unlabeled proteins (fibronectin, transferrin, and IgG) were ineffective. The binding of 125I-fg to ICAM-1-(8–21) peptide was determined using increasing concentration of coating peptide. A representative experimental result is shown from a total of six individual experiments performed for each set.

Specificity of fg-ICAM-1-(8–21) Interaction—The binding of fg to ICAM-1-(8–21) was highly specific. First, under conditions in which 125I-fg bound avidly to ICAM-1-(8–21), other radiolabeled proteins (fibronectin, low density lipoprotein, lipoprotein(a), and IgG) applied at 20–50 nM concentrations did not bind or bound minimally to ICAM-1-(8–21)-coated wells (Fig. 4A). Second, as shown in Fig. 4B, unlabeled fg inhibited the binding of 125I-fg to the peptide, whereas other unlabeled proteins (fibronectin, transferrin, and IgG) were ineffective. For example, unlabeled fg (2.0 μM) inhibited binding of the radiolabeled fg by 86% while fibronectin, at the similar concentration, produced only 12% inhibition. The concentration of fg producing 50% inhibition was in the 0.1–0.2 μM range. Third, R152, a rabbit polyclonal antipeptide antibody directed against ICAM-1-(8–21), blocked the interaction of 125I-fg with immobilized ICAM-1-(8–21). At 10 μg/ml, R152 blocked the binding by 82%, whereas two other unrelated antipeptide antibodies had no effect.

![Image of Table I](http://www.jbc.org/)

**Table I**

| Peptide (sequence) | fg bound (ng) |
|-------------------|---------------|
| ICAM-1-(1–16)     | 0.2 ± 0.06    |
| ICAM-1-(8–20)     | 12.8 ± 1.8    |
| ICAM-1-(8–21)     | 25 ± 3.4      |
| ICAM-1-(8–21) (R→D)| 15.2 ± 1.4    |
| ICAM-1-(9–21)     | 22.8 ± 3.0    |
| ICAM-1-(10–21)    | 12.7 ± 1.3    |
| ICAM-1-(11–21)    | 9.8 ± 1.4     |
| ICAM-1-(16–21)    | 3.5 ± 1.1     |
| ICAM-1-(21–32)    | 0.9 ± 0.2     |
| ICAM-1-(40–52)    | 0.9 ± 0.3     |
| ICAM-1-(70–83)    | 0.9 ± 0.3     |
| ICAM-1-(130–139)  | 0.9 ± 0.3     |
| ICAM-1-(130–145)  | 0.9 ± 0.3     |
| ICAM-1-(152–145)  | 0.9 ± 0.3     |
| ICAM-3-(135–150)  | 0.9 ± 0.3     |
| Integrin β3(783–798)| 0.9 ± 0.3     |
| Integrin β3(1–11)| 0.9 ± 0.3     |
| Integrin αM(446–458)| 0.9 ± 0.3    |
| Integrin αM(1123–1137)| 0.9 ± 0.3 |
| VCAM-1-(1–12)     | 0.9 ± 0.3     |
| VCAM-1-(34–46)    | 0.9 ± 0.3     |
| VCAM-1-(234–246)  | 0.9 ± 0.3     |
R152 was prepared by immunizing rabbits with ICAM-1-(8–21) peptide. The IgG-purified fraction of the antisera reacted specifically with ICAM-1-(8–21) and not with several other ICAM-1 peptides as judged by enzyme-linked immunosorbent assay analysis. The interaction of fg with ICAM-1-(8–21) was divalent cation independent. No differences in the extent of 125I-fg binding were detected using CaCl₂ or MgCl₂ at concentrations of 0–4 mM. Furthermore, addition of 5 mM EDTA did not affect fg binding to ICAM-1-(8–21) (data not shown).

Structure-Function Analyses—In competition studies, we were compelled to use truncated versions of the ICAM-1-(8–21) peptide because the native peptide not only bound but also precipitated soluble fg. As shown in Fig. 5, certain peptides from within ICAM-1-(8–21) competed for 125I-fg binding to immobilized ICAM-1-(8–21). ICAM-1-(11–22), which contains 50% of the ICAM-1-(8–21) sequence, failed to block fg binding. This difference suggested that the hydrophobic COOH-terminal sequence, SVLVTC, ICAM-1-(16–21), may be important for interaction. Indeed, the 6-amino acid peptide was active, although it was less potent than ICAM-1-(11–22).

Serial truncations of the sequence from the NH₂ terminus of ICAM-1-(8–21) caused a sequential decrease in the capacity of these truncated peptides to bind 125I-fg (Table I). Removal of the NH₂-terminal lysine led to only a slight decrease (9%), while deletion of the NH₂-terminal lysine and the adjacent valine resulted in a 49% loss of fg-binding function. The hydrophobic 6-amino acid peptide from the COOH terminus had only 14% residual activity. This value is comparable to the difference between ICAM-1-(11–22) and ICAM-1-(16–21), may be important for interaction. Indeed, the 6-amino acid peptide was active, although it was less potent than ICAM-1-(11–22).

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Fibrinogen Recognition of ICAM-1-(8–21)—The RGD sequences in the Aα-chain and the dodecapeptide (H12) at the extreme COOH terminus of the γ chain are the recognition sequences for the binding of fg to the β₃ integrins, α₅β₃ and α₁β₃ (4, 6). To verify that these β₃ integrin recognition sequences within fg have a role in ICAM-1 binding, representative RGD and γ-chain peptides were tested for their effect on 125I-fg binding to ICAM-1-(8–21). GRGDSP and H12 had no effect (Fig. 4C). R152 was prepared by immunizing rabbits with ICAM-1-(8–21) peptide. The IgG-purified fraction of the antisera reacted specifically with ICAM-1-(8–21) and not with several other ICAM-1 peptides as judged by enzyme-linked immunosorbent assay analysis. The interaction of fg with ICAM-1-(8–21) was divalent cation independent. No differences in the extent of 125I-fg binding were detected using CaCl₂ or MgCl₂ at concentrations of 0–4 mM. Furthermore, addition of 5 mM EDTA did not affect fg binding to ICAM-1-(8–21) (data not shown).

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effect on fg binding to ICAM-1-(8–21), even when used up to 400 μM concentration (Fig. 5). At a substantially lower concentration of 50 μM, these peptides had a profound effect on fg binding to αIIbβ3 and αvβ3 (data not shown), thus verifying their activity (7).

To explore the interaction of fg with the ICAM-1 peptide, fg was digested with plasmin to obtain fragments X, D100, and D80. Fragments D100 and D80 are late plasmin cleavage products when digestion is performed in the presence and absence of Ca2+, respectively (33). Fragments X, D100, and D80 bound selectively to immobilized ICAM-1-(8–21), whereas fragment E reacted poorly (Fig. 6A). When 100 μl of 100 nM solutions of these fragments were applied to immobilized ICAM-1-(8–21) peptide, 39 ng of fg, 27 ng of fragment X, 6.7 ng of fragment D100, and 7.2 ng of D80 bound ICAM-1-(8–21). Thus, the native conformation of fg is important to maintain high capacity ICAM-1-(8–21)-binding activity.

While these studies were in progress, a peptide from within the D80 region was shown to block ICAM-1 expressing cells from binding to fg. To determine whether this sequence is involved in ICAM-1-(8–21) recognition, peptides corresponding to the γ117–133 region and two other peptides from the gamma chain of fg were tested (Fig. 6B). The peptide corresponding to the γ117–133 sequence (NNQKIVNLKEKVALQEA) blocked fg binding to ICAM-1-(8–21) by 82% when used at 400 μM concentration, whereas three other peptides, including one overlapping the COOH-terminal end of γ117–133 (γ124–133) showed no activity. These results indicate that ICAM-1-(8–21) interaction with fg may involve the NH2-terminal sequence within γ117–133 of fg.

Role of ICAM-1-(8–21) Sequence in Cell Adhesion—Assays were performed with Raji cells, a lymphoblastoid B cell line that constitutively expresses levels of ICAM-1 comparable to those of TNF-α-stimulated EC (Fig. 1). Importantly, the expression of αIIbβ3 on these cells is negligible. Because Raji cells grow readily in suspension and do not require cytokines to stimulate ICAM-1 expression, these cells offer an advantage for measuring ICAM-1-related functions. fg binding to Raji was specific and ICAM-1 dependent. Excess unlabeled fg competed 78% of the labeled fg bound, and an anti-ICAM-1 antibody (20 μg/ml) blocked fg binding to Raji by 72% (Fig. 7A). As previously noted, it was not possible to utilize ICAM-1-(8–21) peptide to block soluble fg binding due to precipitation of fg in the presence of peptide; consequently, we sought the use of antipeptide ICAM-1-(8–21) antibody (R152). This antibody, when used at 40 μg/ml, blocked fg binding to Raji by 69%, and at 20 μg/ml it was still capable of inhibiting fg binding by 55%. Normal rabbit IgG had no effect on fg binding. The effect of ICAM-1-(8–21) peptide on the adhesion of Raji cells to immobilized fg was assessed (Fig. 7B). A large component of Raji cell adhesion to fg was mediated through ICAM-1 given that 69% of the adhesion was inhibited by the ICAM-1-specific mAb, LB-2. The expression of other fg-binding integrins such as αvβ3 and αMβ2 on Raji was low by FACS analyses; thus, a small portion of Raji adhesion to fg may be mediated by other unidentified mechanisms. ICAM-1-(8–21) peptide at 200
μM blocked Raji cells adherence to fg by 82% and ICAM-1-(8–20) blocked adhesion by 48%, whereas at the same concentration three control peptides, including VCAM-1-(34–46), had minimal effects. Data for the control peptide ICAM-1-(130–145) are indicated because this peptide is structurally similar to ICAM-1-(8–21); both peptides contain a charged and hydrophilic NH2-terminal portion and a COOH-terminal end which is hydrophobic. Taken together, these results implicate ICAM-1-(8–21) in disrupting fg-ICAM-1 interactions in cellular adhesive processes.

**DISCUSSION**

An interaction involving fg and ICAM-1 results in cellular bridging of monocytic cells and ICAM-1-expressing cells (1). We have demonstrated a direct interaction between fg and intact ICAM-1 and with a truncated two-domain form of ICAM-1 (Fig. 2). Thus, the first two Ig domains of ICAM-1 contain the sites necessary for fg binding. The blockage of fg-mediated Raji adhesion by LB-2, a mAb reported to bind to the first two domains of ICAM-1 (16, 43), further verified that fg interacted with the first two domains of ICAM-1. A sequence within the first Ig domain, ICAM-1-(8–21) (KVILPRGGSVLVTC) was identified that specifically bound fg. Excess unlabeled fg blocked the binding of radiolabeled fg to ICAM-1-(8–21) (Fig. 4B); and peptides containing sequences within ICAM-1-(8–21) were effective in competing fg binding to ICAM-1-(8–21) (Fig. 5). Comparison of ICAM-1-(8–21) sequence in human and murine ICAM-1 reveals that 8 of the 14 residues are identical, while another 4 have conservative substitution. This high degree of homology at ICAM-1-(8–21) is interesting because murine ICAM-1 binds human fg (1). Furthermore, comparison of the ICAM-1-(8–21) sequence within other Ig-like adhesion receptors, such as ICAM-2, ICAM-3, and VCAM-1, reveals amino acid identity only at glycine (Gly-15) and cysteine (Cys-21). However, the COOH end of the sequence is hydrophobic in ICAM-1, -2, and -3 and VCAM-1.

The first two Ig-like domains of ICAM-1 are paired homologues and mediate the interaction with α1β2 and rhinoviruses (16, 17, 44). The first Ig domain of ICAM-1 contains unique residues required for binding α1β2 (28) and rhinoviruses (44), while ICAM-1-(16–19) was implicated in the recognition of erythrocytes infected with the malarial parasite, *Plasmodium falciparum*, by ICAM-1 (43, 45). ICAM-1-(16–19) is contained in the fg recognition sequence [ICAM-1-(8–21)], and since the ICAM-1-(16–19) recognition site on *P. falciparum* is as yet unidentified, it remains to be seen whether the region will have sequence homology with fg, particularly fg117–133. Other linear sequences, ICAM-1-(40–52) and ICAM-1-(367–394), have been reported to block α1β2-ICAM-1-mediated lymphocyte-adhesive functions (21, 46). A sequence within the first Ig domain, ICAM-1-(21–42) was reported to block α1β2-mediated binding to ICAM-2 (47, 48).

Deletion analysis indicated that the intact ICAM-1-(8–21) sequence is required for optimal binding to fg. The COOH-terminal hydrophobic 6-residue peptide (SVLVTC) demonstrated low fg-binding activity, whereas sequential deletion of the first three NH2-terminal residues retained 69% of fg-binding activity (Table I). Furthermore, a substitution of Asp for Arg at position 13 rendered a 39% loss in fg-binding activity. Arg-13 also is predicted to be an important residue from the structural model of ICAM-1-(8–21) (Fig. 8), and point mutation of this and other residues within the ICAM-1-(8–21) will establish the importance of these residues. These studies, however, further validate the specificity of fg binding to ICAM-1-(8–21) and indicate that fg binding to ICAM-1 is not simply a hydrophobic interaction.

The fg-binding activity of ICAM-1-(8–21) was not due to dimerization, because HPLC analysis of ICAM-1-(8–21) in the presence and absence of the reducing agent (dithiothreitol) indicated that ICAM-1-(8–21) did not exist in a dimeric state. In addition, peptide ICAM-1-(8–20) lacking the cysteine residue also bound fg, but less avidly than ICAM-1-(8–21). These results indicate that the cysteine residue is important in perhaps stabilization of the overall ICAM-1-(8–21) structure, but not for dimerization.

Secondary structure analyses of ICAM-1-(8–21) indicated that the amino terminus is hydrophilic, and the COOH end is predicted to be hydrophobic. The NH2-terminal region of ICAM-1-(8–21) lies in β-strand A and forms a loop involving the two glycine and single proline residues (12). The COOH half, including the hydrophobic residue from the β-strand B, is predicted to be partially buried and offering rigidity to the intact molecule. We modeled the first Ig domain of ICAM-1 using coordinates reported for the crystal structure of VCAM-1 (36). The NH2-terminal residues are solvent exposed, and peptide deletion experiments (Table I) confirm the importance of these residues in fg binding. The ICAM-1-(8–21) sequence may not be fully exposed in the intact protein (Fig. 8) which may explain why the binding of fg to intact ICAM-1 appeared to be of lower affinity compared with the binding of fg to ICAM-1-(8–21). It is likely that exposure of the ICAM-1-(8–21) region may be regulated by either ICAM-1 activation or receptor rearrangement for optimal binding of fg to ICAM-1-expressing cells.

ICAM-1-(8–21) bound to the D80 fragment of fg. This result is consistent with our observation that the GRGDSP peptide was ineffective in blocking fg binding to ICAM-1-(8–21). Hatzfeld et al. (49, 50) noted fragment D but not an RGD-containing peptide to compete for fg binding to a mitogenic fg receptor on Raji. The fg-γ chain sequence (117–133) which was recently shown to block fg binding to ICAM-1-expressing cells (51) also blocked fg binding ICAM-1-(8–21), indicating that...
these two sequences may be the reactive pairs in fg-ICAM-1 interaction.

Cellular assembly involving fg is reported to be vital in the recruitment of inflammatory cells onto biomaterial implants in animal models (52, 53). fg-ICAM-1 association is implicated in cellular transmigration (19), and recently, contraction of endothelium was reported to be in part mediated by fg interaction with ICAM-1 (54). However, whether the ICAM-1-(8–21) peptide is capable of blocking leukocyte transmigration and endothelial contraction remains to be investigated. We have noted that fg-bound platelets bridge via ICAM-1 on EC. The participation of ICAM-1-(8–21) and fg-(117–133) in platelet-EC interaction is under investigation in an effort to understand the role of platelets in inflammatory processes, such as atherosclerosis.

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