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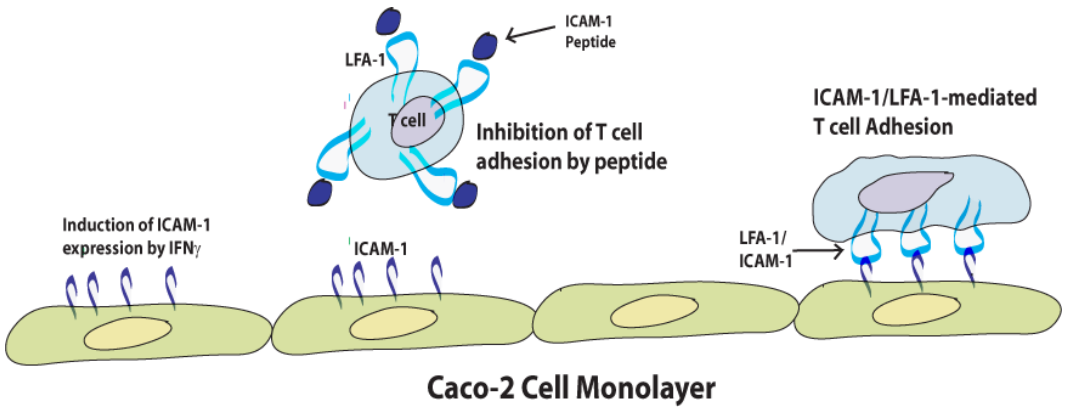
Abstract:

In this work, we have designed cyclic peptides (cIBL, cIBR, cIBC, CH4 and CH7) derived from the parent IB peptide (ICAM-$1_{1–21}$) that are inhibitors of ICAM-1/LFA-1-mediated T-cell adhesion to Caco-2 cell monolayers. Cyclic peptide cIBR has the best activity of any of the peptides evaluated. The active ICAM-1 peptides have a common Pro-Arg-Gly sequence that may be important for binding to LFA-1.

Graphic Abstract:

Inhibition of LFA-1/ICAM-1-mediated Tcell adhesion by ICAM-1-derived peptides



Text of paper:

Inhibition of ICAM-1/LFA-1-mediated Heterotypic T-cell Adhesion to Epithelial Cells: Design of ICAM-1 Cyclic Peptides

Meagan E. Anderson, Tatyana Yakovleva, Yongbo Hu, and Teruna J. Siahaan*

Department of Pharmaceutical Chemistry, The University of Kansas, Simons Research Laboratories, 2095 Constant Avenue, Lawrence Kansas 66047

M.E. Anderson, T. Yakovleva, Y. Hu, and T. J. Siahaan, "Inhibition of ICAM-1/LFA-1-mediated heterotypic T-cell adhesion to epithelial cells: Design of ICAM-1 cyclic peptides," Bioorg. Med. Chem. Lett. 14, 1399–1402 (2004). [PMID: 15006370]. Publisher's official version: http://dx.doi.org/10.1016/j.bmcl.2003.09.100. Open Access version: http://kuscholarworks.ku.edu/dspace/.

Autoimmune diseases are characterized by the activation and migration of 'self'-reactive' T-cells to target antigens in a specific tissue or organ.[1] The process of T-cell migration from the blood stream to tissues involves the interaction between lymphocyte associated antigen-1 (LFA-1) and intercellular adhesion molecule-1 (ICAM-1).[2] T-cell adhesion to vascular endothelial cells is mediated by the activated form of the LFA-1 integrin receptor. The activated form of LFA-1 can be induced *in vivo* by inflammatory cytokines and *in vitro* by anti CD-2 antibody or phorbol esters.[3,4] This facilitates T-cell extravasation through vascular cell junctions into tissues containing the site of the autoimmune reaction and inflammation.

Table 1. Sequences of ICAM-1-derived peptides.

Name	Amino Acid Sequence
IB	QTSVSPSKVILPRGGSVLVTG
cIBL	Cyclo(1,12)Pen-QTSVSPSKVIC[a]
cIBC	Cyclo(1,12)Pen-PSKVILPRGGC[a]
cIBR	Cyclo(1,12)Pen-PRGGSVLVTGC[a]
CH4	Cyclo(1,6)VILPRG[b]
CH7	Cyclo(1,6)PRGGSV[b]
CHK7	Cyclo(1,6)PRGGKV[b]

[a]Cyclic peptides were formed by disulfide bond from Pen1 to Cys12. [b]These peptides were cyclized by an amide bond.

Many therapies are currently being developed to treat the progression of autoimmune disease by modulating the interaction of LFA-1/ICAM-1. For example, anti-ICAM-1 and anti-CD11a monoclonal antibodies (mAb) have been shown to inhibit T-cell adhesion mediated by LFA-1 and have been developed for the treatment of rheumatoid arthritis, insulin-dependent diabetes, and psoriasis.[5,6] Also, small organic molecules such as lovastatin,[7] *p*-arylthiocinnamides, and hydantoin derivatives (i.e., BRIT-377)[8,9] have been shown to inhibit ICAM-1/LFA-1 interactions by binding to the I-domain of LFA-1.

In this work, we designed cyclic peptides that are derived from the parent linear peptide IB (ICAM-$1_{1–21}$) (Table 1). The sequence of the linear IB peptide was derived from ICAM-1, residues 1–21 of the Domain 1 (D1) N-terminus. The ability of peptides to inhibit ICAM-1/LFA-1 interaction was evaluated using a heterotypic cell adhesion assay between a human leukemia T-cell line (Molt-3) and Caco-2 epithelial cell monolayers, which are a model for the intestinal mucosa.[10,11] This experimental model of T-cell adhesion to intestinal mucosa is relevant to autoimmune diseases such as Crohn's disease and ulcerative colitis.[2,12]

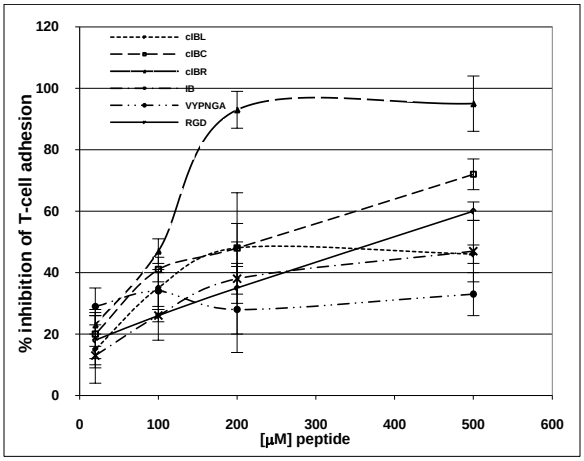


Figure 1. Concentration-dependent activity of ICAM-1 peptides in heterotypic T-cell adhesion assay.

It is apparent that cIBR is the strongest inhibitor of T-cell adhesion at all concentrations (Figure 1). At 500 μM cIBC is the second-most active peptide, followed by the cIBL and IB peptides, which are nearly equivalent. This result is consistent with previous findings in which the affinity of the cyclic peptides to the isolated LFA-1 receptor was also in the following order: cIBR>cIBC>cIBL.[13] These results suggest that, for cIBR and cIBC, the conformational restriction induced by cyclization may contribute to their binding affinity to LFA-1 and ability to inhibit T-cell adhesion. Both cIBR and cIBC peptides contain β-turn-like structures around this PRG sequence as determined by NMR.[14,15] This sequence is representative of the β-hairpin turn that connects the A' and B β-strands in the D1 domain of ICAM-1.[16] The negative control, VYPNGA, demonstrated no concentration-dependent activity. The positive control RGD tripeptide demonstrated a concentration-

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dependent ability to inhibit T-cell adhesion, presumably by inhibiting other integrins. The cIBR peptide was a distinctly better inhibitor of T-cell adhesion than was the RGD control, suggesting that the ICAM-1/LFA-1 interaction is essential for the adhesion process. This indicates that LFA-1-mediated adhesion is the preferred mechanism utilized by T-cells to adhere to epithelial cells.

The binding sites of cyclic peptides cIBL, cIBC, and cIBR were previously mapped to the I-domain of LFA-1 and at a region partially overlapping with the binding site of anti-CD11a mAb R3.1 binding site.[13] To demonstrate that ligand binding to this region of the I-domain of LFA-1 inhibits T-cell adhesion to Caco-2 monolayers, the anti-LFA-1 antibody R3.1 was also used as a positive control (Figure 2). The R3.1 antibody inhibited the adhesion of T-cells to monolayers in a dose-dependent manner, suggesting that the ability of these peptides to inhibit T-cell adhesion was due to their binding to this region on the I-domain (Figure 2).

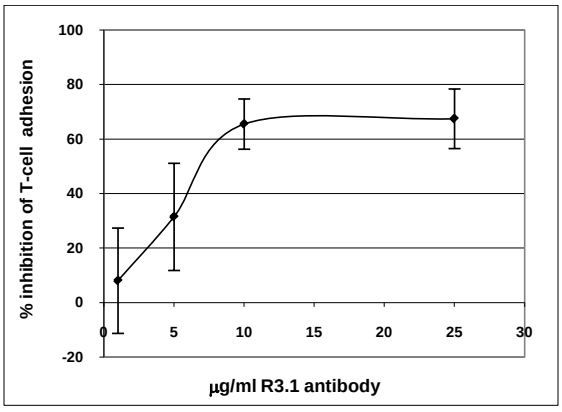

Figure 2. Concentration-dependent inhibitory activity of LFA-1 mAb R3.1 in heterotypic T-cell adhesion assay.

To evaluate the importance of the Pro-Arg-Gly sequence and to reduce the size of the cyclic peptide, we synthesized a series of linear hexapeptides LH1–LH8, which have over-lapping sequences derived from the cIBC and cIBR peptides (Table 2). These peptides were evaluated at 200 μM for their ability to inhibit T-cell adhesion (Table 2). Among these linear hexapeptides, LH4 (VILPRG) and LH7 (PRGGSV) were found to be the most effective inhibitors of T-cell adhesion. Both peptides contain a Pro-Arg-Gly (PRG) sequence, also found in cyclic peptides cIBR and cIBC. These results suggest that the sequence and structure around this PRG motif are important for the activity of these peptides.

Table 2. Linear hexapeptides derived from the sequences of cIBR and cIBC

Name	Sequence	% T-cell Binding[a]
LH1	PSKVIL	44 ± 4
LH2	SKVILP	*
LH3	KVILPR	49 ± 5
LH4	VILPRG	32 ± 3
LH5	ILPRGG	46 ± 4
LH6	LPRGGS	37 ± 4
LH7	PRGGSV	25 ± 1
LHA1	AILPRG	53 ± 2
LHA2	VALPRG	71 ± 2
LHA3	VIAPRG	46 ± 2
LHA4	VILARG	62 ± 1
LHA5	VILPAG	*
LHA6	VILPRA	68 ± 2

[a] Each peptide was used at a concentration of 200 μM in the cell adhesion assay. *This peptide was not evaluated due to its instability.

The linear hexapeptide LH4 was subjected to alanine scanning to give linear hexapeptides LHA1–LHA6 (Table 2). Alanine substitution of Ile2, Pro4, or Gly6 residues reduced the activity of the peptide relative to LH4. This suggests that these residues play an essential role in the ability of these peptides to recognize LFA-1 and inhibit T-cell adhesion.

To evaluate the effect of the conformation of peptides on inhibition of T-cell binding to Caco-2 monolayers, cyclic hexapeptides CH4 and CH7, which were derived from the most effective linear hexapeptides LH4 and LH7, were synthesized (Table 1). The formation of cyclic hexapeptides has been shown to stabilize the β-turn structure and improve chemical and enzymatic stability. Therefore, the formation of CH4 and CH7 is expected to stabilize the β-turn

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at their Pro-Arg-Gly sequence as in cIBC and cIBR peptides.[15,14] Cyclic peptide CH7 has activity similar to that of the linear LH7 (Figure 3). However, cyclic hexapeptide CH4 demonstrated lower activity than its linear analog LH4. These results suggest that both conformational restriction and amino acid sequence influence the activity of these peptides. This difference in activity may be due to changes in the conformation of the backbone and the side chains of PRG sequence upon cyclization. In addition, cyclization may alter the ratio of *cis* to *trans* conformation at the Xaa-Pro peptide bond in CH4 and CH7, influencing their activity.

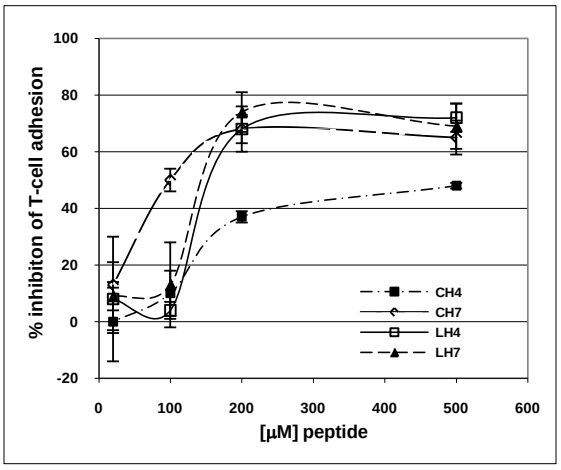


Figure 3. Concentration-dependent activity of linear peptides LH4 and LH7, and cyclic peptides CH4 and CH7.

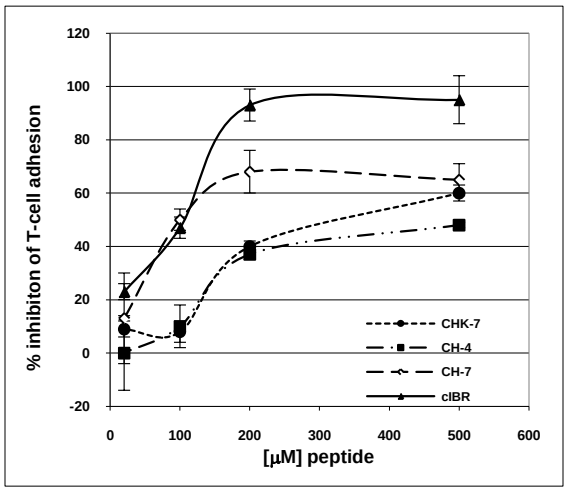
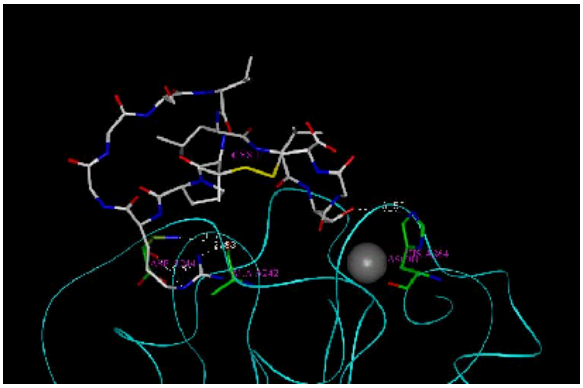


Figure 4. Concentration-dependent activity of cyclic peptide CH7K compared to that of cyclic peptides cIBR, CH4 and CH7.

This cyclic peptide CH7 may be developed as a targeted drug delivery system to LFA-1-expressing T-cells.[17] To accommodate drug conjugation, a Lys residue was incorporated by replacing Ser5 with Lys to give peptide CHK7 (Table 1). Compared to CH7, the CHK7 peptide demonstrated lower activity, which suggests that the Ser5 residue is important for activity (Figure 4). Figure 4 also shows that cIBR has the best inhibitory activity compared to the cyclic hexapeptides (CH4, CH7 and CHK7). In the future, other residues in CH7 will be substituted



with Lys to find a derivative that maintains its LFA-1-binding activity.

Figure 5. Molecular docking the cIBR peptide to the I-domain of LFA-1 at the metal ion-dependent adhesion site (MIDAS).

The binding sites of the cyclic and linear peptides on the I-domain of LFA-1 were evaluated using docking experiments.[18,19] Previously using monoclonal antibody inhibition assay, we have determined that these peptides bind to the I-domain of LFA-1.[13] Docking studies of the cyclic peptides (cIBR, cIBC, and cIBL) reveal that these cyclic peptides have overlapping binding sites on the I-domain of LFA-1. Figure 5 shows that cIBR peptide binds at the groove region of the I-domain. Both of cIBR and cIBC peptides form hydrogen bonds to several residues on the I-domain, including Ala242, Asp244, Gln266, and Glu269. On the

other hand, cyclic peptide cIBL binds to the I-domain around residues 143–149 close to the α_4-helix. The linear peptides LH4 and LH7 were predicted to bind to the I-domain between the α_3-β_5 loop and α_4-helix, which was similar to the binding site of cyclic peptide cIBL.

Our results suggest that the active ICAM-1 peptides have a common PRG sequence, which may be important for binding to LFA-1. Interestingly, another β_2 integrin ($\alpha_x\beta_2$) was found to have a similar proline-containing recognition sequence, GPRP, which mediates rotavirus adhesion.[20] Proline is known to stabilize β-turns. Here, Pro may stabilize the conformation of the linear peptides' PRG motif, facilitating their recognition by the LFA-1 β_2-integrin. This has been demonstrated in RGD peptides with flanking proline residues, which help to maintain a favorable peptide conformation for recognition by β_1- and β_3-integrins.[21]

In conclusion, we have reduced a large ICAM-1 peptide (IB) to a cyclic hexapeptide (CH7) that can bind to LFA-1 and inhibit T-cell adhesion in a concentration-dependent manner. The PRG motif may be important for the activity of these peptides. The cyclic peptide CH7 will be used to design peptidomimetics that can bind to the I-domain of LFA-1. In addition, the cyclic hexapeptide CH7 will be optimized for targeting drugs to leukocytes. The studies presented here may ultimately lead to the identification of an optimal peptide for designing small peptidomimetics that retain the activity of these ICAM-1-derived peptides.

ACKNOWLEDGMENTS

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19. Docking calculations were performed with the program AutoDock (version 2.4).18 The crystal structure of LFA-1 I-domain Protein Data Bank (PDB) database entry 1LFA was used as the target for the docking simulations of various peptides.16 For cIBR and cIBC, the initial searching structure was the NMR structure of these cyclic peptides, which was previously determined.14,15 The coordinates of initial searching structures of linear peptides LH4 and LH7 were adapted from the coordinates of the corresponding sequences in the crystal structure of ICAM-1 (PDB entry 1IAM).16 The affinity grid of the target protein LFA-1 I-domain was generated first with the auxiliary program AutoGrid. The position of Ca^{2+} is chosen as the center of the grid because mAb inhibition and Ca^{2+}-dependent binding studies suggest that the binding region of cIBR peptide is at the metal ion-dependent adhesion site (MIDAS) of the I-domain. The dimensions of the grid were 50.0 × 50.0 × 50.0 Å with grid points separated by 0.5 Å. All dihedral angles that can be rotated in the peptide side chains were assigned with the auxiliary program AutoTors and were allowed to rotate freely. A total of 128 runs were performed for each peptide and each simulation run lasted 110 cycles with a starting temperature of 616 K in the first cycle and a temperature reduction factor of 0.95 per cycle. The lowest energy structures produced by AutoDock runs were taken as the possible binding models.
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