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Cell Surface-Expressed Moesin-Like Receptor Regulates T Cell Interactions with Tissue Components and Binds an Adhesion-Modulating IL-2 Peptide Generated by Elastase

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The adhesion of leukocytes to the extracellular matrix (ECM) depends on their responses to variations in the chemotactic signals in their milieu, as well as on the functioning of cytoskeletal and context-specific receptors. Ezrin, radixin, and moesin constitute a family of proteins that link the plasma membrane to the actin cytoskeleton. The surface expression of moesin on T cells and its role in cell adhesion has not been fully elucidated. Recently, we found that IL-2 peptides generated by elastase modified the adhesion of activated T cells to ECM ligands. Here, we further examined the adhesion regulatory effects of EFLNRWIT, one of the IL-2 peptides, as well as the existence and putative function of its receptor on T cells. We found that when presented to T cells in the absence of another activator, the EFLNRWIT peptide induced cell adhesion to vessel wall and ECM components. Binding of a radiolabeled peptide to T cells, precipitation with the immobilized peptide, and amino acid sequencing of the precipitated protein revealed that EFLNRWIT exerts its function via a cell surface-expressed moesin-like moiety, whose constitutive expression on T cells was increased after activation. This notion was further supported by our findings that: 1) anti-moesin mAb inhibited the binding of T cells to the immobilized EFLNRWIT peptide, 2) immobilized recombinant moesin bound the IL-2 peptide, and 3) soluble moesin inhibited the EFLNRWIT-induced T cell adhesion to fibronectin. Interestingly, moesin appears to be generally involved in T cell responses to adhesion-regulating signals. Thus, the IL-2 peptide EFLNRWIT appears to exert its modulating capacities via an adhesion-regulating moesin-like receptor. The Journal of Immunology, 2001, 166: 3052–3060.

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3 Abbreviations used in this paper: ECM, extracellular matrix; HA, hyaluronic acid; FN, fibronectin; LN, laminin; CO, collagen; MIP-1β, macrophage-inflammatory protein-1β; KLH, keyhole limpet hemocyanin; MS, mass spectrometer; ERM, ezrin, radixin, moesin.
effects of the peptides depended on their interactions with a moe-
sin-like receptor expressed on the surface of T cells; blockage of
moesin inhibited T cell adhesion to tissue ligands. We discuss the
possibility that the behavior of T cells in inflamed areas is affected
by receptor-specific recognition of the molecular breakdown prod-
ucts of the very signals that elicit the inflammatory reaction.

Materials and Methods

Human rIL-2 (Chiron B.V., Amsterdam, The Netherlands); macrophage-
inflammatory protein-1β (MIP-1β; PeproTech, Rocky Hill, NJ); PMA,
PHA, keyhole limpet hemocyanin (KLH), BSA, and HA (Sigma, St. Louis,
MO); FN (Chemicon, Temecula, CA); LN, CO type IV (ICN Pharmaceut-
icals, Costa Mesa, CA); calcyulin A (Alexis Biochemicals, San Diego,
CA); and antibiotics and RPMI 1640 (Beit-Haemek, Israel) were obtained
as indicated. The mouse anti-human moesin mAb clone 38/87 was obtained
from NeoMarkers (Fremont, CA); mAb anti-CD3 (clone UCH-T1; Pharm-
ingen, San Diego, CA), -anti-CD69 (clone CH4), -anti-CD49e (VLA-5,
clone JB5S), CD29 (clone 3S3), CD44 (clone 5035-41.1D), and -LFA-1
(CD11a, clone 121/7) were obtained from Serotec (Oxford, U.K.), and
FITC-conjugated rabbit anti-human Ab from Dako (Glostrup, Denmark).
CD29 (clone JBS5), CD29 (clone 3S3), CD44 (clone 5035-41.1D), and
-CD44 (all 1 µg/ml), and Ig-conjugated ICAM-1, and VCAM-1 (5 µg/ml).
The remaining binding sites were blocked with 0.1% BSA. 125I-labeled T cells (106 cells in 100
µl of adhesion medium (RPMI 1640 containing antibiotics and 0.1% BSA)
(21) were then added to the coated wells followed by the addition of IL-2,
PHA, or KLH-conjugated IL-2 peptides. In some experiments, soluble recombi-
nant moesin had been preincubated (240 min, 37°C) with IL-2 or the IL-2
peptide and added to the wells with the T cells. Where indicated, T cells
were pretreated with mAb anti-moesin (clone 38/87), anti-LFA-1, anti-
CD29, and anti-CD44 (all at 1 µg/ml), and then activated by various ac-
tivators. The microrotter plates were then incubated (37°C in a 7.5% CO2
humidified atmosphere) for 60 min for assaying T cell adhesion to FN,
ICAM-1, and VCAM-1, and for 3 h for assaying T cell adhesion to HA. At
the conclusion of the incubation periods, the microrotter plates were gently
washed with warm PBS, the adherent cells were lysed with 1% Tween
20 in 1 N NaOH, and the radioactivity associated with the resulting superna-
tants was determined using a gamma counter. For each experimental group,
the results were expressed as the mean percentage (± SD) of bound T cells
from quadruplicate wells. The percentage of cells that adhered was calcu-
lated as follows: [cpm of residual bound cells in the well/(total cpm of cells
in the well)/total cpm of cells added to the well] × 100.

Peptide binding assays

The EFLNRWIT-AY peptide was iodinated with 125I using the chloramine
T labeling method (9). The labeled peptide was isolated from the solution
using a Sephadex G-10 column (Pharmacia Biotech, Uppsala, Sweden).
The specific activity of the125 I-labeled peptide thus obtained was 0.6
µCi/µg peptide. The labeled peptide was incubated (40 nM; 4°C, 90 min)
with Jurkat cells (106) in the presence of increasing amounts of unlabeled
EFLNRWIT, RMLT, and JVL or TlVRNLFs peptides. The cells were
then washed three times with cold PBS, and the radioactivity associated
with the cells was determined using a gamma counter. In other experi-
ments, KLH or KLH-conjugated IL-2 peptides (0.25 µg/well) were
immobilized (18 h, 4°C) on microtiter plates (Maxisorp, Nunc, Denmark).
The unbound areas of the wells were blocked with KLH (1 µg/well), 1 h,
25°C). 125I-labeled Jurkat cells (106 cells/well) were then added to the
plates and incubated (4 h, 4°C) in the presence of anti-moesin or anti-CD3
mAb. The plates were then washed with PBS, the adherent cells lysed, and
the amount of radioactivity in the resulting lysates was measured.

Protein precipitation and Western blotting

Affi-Gel 10 beads were washed with MOPS (0.1 M, pH 7.5) and incubated
(18 h, 4°C) with KLH (100 µg) or KLH-conjugated IL-2 peptides (100 µg)
in 500 µl of MOPS containing 40 mM CaCl2. These KLH-coupled beads
were washed with PBS and loaded with precleared lysates of Jurkat cells
(106 cells per lane). The beads were lysed by incubating them with H2O
containing sucrose (0.15 M), β-glycerophosphate (80 mM), EDTA (2
mM), EGTA (2 mM), NaVO3 (10 mM), Triton X-100 (1%), pepstatin (10
µg/ml), leupeptin (10 µg/ml), and PMSF (2 mM). Next, the lysates of the
T cells were centrifuged (15 min, 15000 rpm); the resulting supernatant
was collected and preincubated with KLH-coupled beads (1 h, 4°C). Finally,
the beads were precipitated by centrifugation and the supernatant was
collected and incubated (4 h, 4°C) with the KLH-conjugated EFLNRWIT
beads or KLH beads as controls. In some experiments, T cell lysates were
immobilized (30 min, 4°C) with soluble EFLNRWIT (400 µg/ml), TlVRN

PepTIDEs and T cell adhesion assays

Human T cells were purified from the peripheral blood of healthy donors
as previously described (4, 10, 11). Briefly, human leukocytes were iso-
atcd on a Ficoll gradient, washed, and incubated (2 h, 37°C, 7.5% CO2,
humidified atmosphere) on petri dishes. The nonadherent cells were then
cooled and incubated (1 h, 37°C, 7.5% CO2, 3 ml) in 3 ml of RPMI
1640 on nylon wool columns (Novamed, Jerusalem, Israel). Unbound cells
were eluted from the columns by extensive washings. The resulting cell popu-
lation was always >90% T cells, as determined by FACS analysis. The
Jurkat T cell clone, which consists of CD4+ leukemic T cells, was used in
some experiments.

T cell adhesion to immobilized protein substrates was also examined as
previously described (11). Briefly, flat-bottom microtiter plates were pre-
coated with FN, LN, CO-IV (all at 0.5 µg/well), HA (5 µg/well), and
Ig-conjugated ICAM-1, and VCAM-1 (5 µg/well). The specific activity of the125 I-labeled peptide thus obtained was 0.6
µCi/µg peptide. The labeled peptide was incubated (40 nM; 4°C, 90 min)
with Jurkat cells (106) in the presence of increasing amounts of unlabeled
EFLNRWIT, RMLT, and JVL or TlVRNLFs peptides. The cells were
then washed three times with cold PBS, and the radioactivity associated
with the cells was determined using a gamma counter. For each experimental group,
the results were expressed as the mean percentage (± SD) of bound T cells
from quadruplicate wells. The percentage of cells that adhered was calcu-
lated as follows: [cpm of residual bound cells in the well/(total cpm of cells
in the well)/total cpm of cells added to the well] × 100.

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mM), EGTA (2 mM), NaVO3 (10 mM), Triton X-100 (1%), pepstatin (10
µg/ml), leupeptin (10 µg/ml), and PMSF (2 mM). Next, the lysates of the
T cells were centrifuged (15 min, 15000 rpm); the resulting supernatant
was collected and preincubated with KLH-coupled beads (1 h, 4°C). Finally,
the beads were precipitated by centrifugation and the supernatant was
collected and incubated (4 h, 4°C) with the KLH-conjugated EFLNRWIT
beads or KLH beads as controls. In some experiments, T cell lysates were
immobilized (30 min, 4°C) with soluble EFLNRWIT (400 µg/ml), TlVRN

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Expression in bacteria and purification of recombinant human moesin

Recombinant human (full-length) moesin was expressed in Escherichia coli as a fusion protein with GST. E. coli transfected with the pGluMo plasmid (pGEX-KG-human moesin residues 1–577, or GST-moesin) (kindly provided by Dr. Furthmayr, Stanford, CA) was grown in t-broth containing ampicillin. The expression of fusion protein was induced with 100 \mu M isopropyl \( \beta \)-thiogalactopyranoside. Recombinant GST-fusion protein was bound to a glutathione-agarose (Sigma) column and cleaved with thrombin (Pharmacia, Piscataway, NJ) as previously described (12, 13). The purified moesin protein thus obtained was dialyzed against PBS (at 4°C) and stored at –70°C. The purity and integrity of the recombinant moesin was determined and confirmed by SDS-PAGE and Western blotting with the anti-moesin mAb clone 38/87. The amount of recombinant moesin was measured by the densitometry of Coomassie stained SDS-PAGE of the moesin and known amounts of BSA, which were subsequently used to construct a standard curve.

FACS analysis of the expression of moesin

We determined the cell surface expression of moesin on freshly purified human PBL (isolated and purified as previously described) (4) after their stimulation with different stimulators. The cells were maintained in RPMI 1640 medium containing 1% HEPES, 1% l-glutamine, sodium pyruvate (200 mM), and 10% FCS and antibiotics (Kibbutz Beit-Haemek, Israel). Where indicated, the T cells were activated (48 h in tissue culture conditions), PHA (10 ng/ml), PHA (8 \mu g/ml), or calyculin A (100 nM). All staining protocols were performed with staining buffer (PBS/0.1% BSA and 0.01% sodium azide). For direct staining, the freshly purified human T cells were stained (45 min, 4°C) with PE-conjugated anti-CD3 mAb (lot diluted 1:2000). The immunoblots were subjected to autoradiography, and the autoradiograms were scanned.

Results

Induction of T cell adhesion to ECM components and endothelial cell ligands by the EFLNRWIT peptide

Previously, we showed that the peptides IVL, RMLT, and EFLNRWIT, generated from IL-2 by elastase, inhibit the adhesion of IL-2- and PMA-activated T cells to FN, LN, and CO type IV (4). Thus, the IL-2 peptides manifest an anti-adhesive effect if the targeted T cells are coincidentally exposed to proinflammatory signals. However, while migrating, T cells may encounter cleaved IL-2 peptides in the absence of an adhesion- or migration-strengthening stimulus in the form of chemokines or cytokines. Therefore, we first elucidated the effects of the IL-2 peptide, EFLNRWIT, in the absence of other stimuli, on T cell adhesion to ECM glycoproteins and molecular components of blood vessel walls. When T cells were exposed to the IL-2 peptide alone, a substantial cellular adhesion was observed not only to immobilized FN, LN, CO-IV, and HA (Fig. 1 A), but also to the bound vessel wall molecules, ICAM-1 and VCAM-1 (Fig. 1 B). The EFLNRWIT-induced T cell adhesion to VCAM-1 was greater than that to ICAM-1. Note that T cell adhesion to FN, LN, CO, as well as to ICAM-1 and VCAM-1 was induced by relatively low amounts (0.001–0.1 pg/ml) of EFLNRWIT, but this slowly decreased when higher concentrations were used. However, with HA, exposure of the T cells...
to increasing amounts of the peptide resulted in a marked and consistent increase of T cell adhesion to the ligand, with maximal adhesion occurring at 1 to 10 pg/ml. A similar pattern of results was obtained with Jurkat cells, a CD4+ T cell clone. The control peptide TIWRNLFE, used in equal concentrations, had no apparent effect on T cell adhesion to the various ligands. Adhesion of T cells to BSA-coated microtiter wells, which were used as a control, was not affected by the IL-2 peptide (data not shown).

The modulation of the observed adhesion was peptide and sequence specific; substituting the two N-terminal amino acids of EFLNRWIT with EA or AF abrogated the effectiveness of the molecule, and the reversely synthesized peptide (TIWRNLFE) did not induce the adhesion of T cells to FN (data not shown). It is noteworthy that the IL-2 peptide-induced T cell adhesion to FN and HA was specifically blocked by mAb anti-αβ1 and anti-αβ2 integrins, or anti-CD44, respectively, but not by control mAb. Furthermore, the peptide-induced T cell adhesion to ICAM-1 was inhibited by anti-LFA-1, but not by anti-CD44 or β2 integrin mAb (data not shown). Thus, depending on the amounts of the IL-2 peptides and the absence or presence of other adhesion-inducing mediators, these peptides can induce (Fig. 1) or inhibit (4) T cell adhesion not only to ECM ligands, but also to ICAM-1 and VCAM-1.

Specificity of the binding of the IL-2 peptide EFLNRWIT to Jurkat T cells

The results of our previous study (4) and of the foregoing experiments suggest that the elastase-generated IL-2 peptide EFLNRWIT exerts its immuno-modulatory functions by interacting with a T cell surface-expressed receptor (other than the IL-2R) (4). This possibility was examined by adding two amino acids, A and Y, to the C-terminal end of the EFLNRWIT peptide, radioactively labeling the modified peptide, and adding it to the Jurkat T cells together with increasing amounts of unlabeled EFLNRWIT or its reversely synthesized counterpart. The two other unlabeled elastase-generated IL-2 peptides, i.e., IVL and RMLT, were also used to compete on the binding of EFLNRWIT to the T cells. Human Jurkat T cells were chosen based on their clonotype characteristics (e.g., durability, cell-to-cell similarity, and accessibility), upon verifying their positive response to the adhesion-promoting amounts of the EFLNRWIT peptide. The amount of peptide that bound to the T cells was plotted and the binding characteristics and specificity of the receptor-ligand interactions were analyzed. The binding curve indicates that the EFLNRWIT peptide specifically bound to the T cells; the unlabeled EFLNRWIT, but not the TIWRNLFE peptide competed, in a dose-dependent manner, with the binding of the labeled peptide to the cells (Fig. 2). Interestingly, competition was not observed with soluble IVL and RMLT peptides, suggesting that although the three peptides exert comparable effects on T cells (4), they may interact with distinct cell surface binding sites or receptors. Moreover, the pattern of the displacement curve revealed that the EFLNRWIT peptide interacts with two distinctive binding sites on the human Jurkat cells, with an estimated EC50 of 9.7 × 10^-9 M for one binding site and 2.5 × 10^-6 M for the other. Thus, the IL-2 peptides exert their functions on T cells by binding to cell surface-expressed moieties with medium-to-high binding affinities.

Moesin was identified as the IL-2 peptide-binding moiety on T cells

The chemical nature of the putative IL-2-peptide binding moiety was examined by lysing Jurkat T cells, preclearing the cell lysate with KLH-coupled beads, and incubating the unbound material with beads coupled with either KLH or EFLNRWIT conjugated to KLH. The unbound material (soluble proteins) was washed, and the precipitated proteins were subjected to SDS-PAGE, and stained for proteins. A major protein band, of an apparent molecular mass of 78 kDa, was specifically precipitated from Jurkat lysates with KLH-EFLNRWIT-conjugated beads (Fig. 3A). The binding of KLH-EFLNRWIT to the 78-kDa protein was substantially reduced in the presence of soluble EFLNRWIT, but not by the reversed soluble peptide or KLH (see the densitometric analysis of Fig. 3A).

Next, the 78-kDa protein band was removed from the gel and a peptide-sequencing analysis of different protein fragments of the molecule was performed. The resulting amino acid sequence was found to be identical with that of the ezrin, radixin, moesin (ERM) protein moesin implicated in cell adhesion (15, 16). Whether moesin is indeed precipitated by the IL-2 peptide was verified by using the anti-moesin mAb clone 38/87 in Western blot analysis. The major moiety in the cell lysates that were bound (precipitated) by the EFLNRWIT molecule was clearly recognized by the anti-moesin mAb (Fig. 3B). Although mAb 38/87 also recognizes radixin, it is highly unlikely that this is the precipitated molecule, because T cells do not express radixin (15).

Analysis of the surface expression of moesin on resting and activated T cells

Can an ERM moesin-like molecule be expressed on the surface of human T cells? Moesin has been characterized mainly in the interior parts of different types of cells. Recently, however, the expression of a molecule identical with moesin on the membrane of leukocytes and other cell types was demonstrated (7, 8, 17–19). Therefore, we examined the expression of moesin on the membrane of resting or activated T cells. However, as seen in Fig. 4, the activation of the T cells for 48 h by PHA and PMA, induced a pronounced elevation (i.e., 2-fold and 4-fold, respectively; Fig. 4E) in the expression of this molecule above the control. As expected, if the membranes of the T cells were permeabilized before adding the mAb anti-moesin, a substantial amount of positive staining occurred (data not shown), indicating
that the mAb anti-moesin used here indeed interacts with the internal ERM molecule.

It has recently been shown that calyculin A, a selective inhibitor of Ser/Thr phosphatase 1 and 2A, can phosphorylate and subsequently induce the association of moesin with F-actin and its redistribution in the cytoplasms of platelets and macrophages (20). Therefore, the effect of PBL treatment with calyculin A on the cell surface expression of moesin was examined. The results, shown in Fig. 4C, indicate that the expression of moesin on T cells treated with calyculin A (100 nM, 48 h) was increased by almost twofold over the control (Fig. 4E). Thus, it appears that resting, and to a much greater degree, activated human T cells express moesin on their cell surfaces.

**Interaction of the IL-2 peptide EFLNRWIT with recombinant human moesin**

The results of the foregoing studies indicate that the EFLNRWIT peptide binds to a moesin-like molecule, or alternatively, to a moesin-associated molecule on T cells. This assumption was further examined, on the molecular level, in the following set of experiments.

First, we assumed that if indeed the IL-2 peptide binds a T cell surface-expressed moesin, then anti-moesin mAb would be expected to competitively inhibit the binding of the peptide to T cells. Such competition was studied by analyzing the binding of labeled human T cells to immobilized KLH-conjugated EFLNRWIT in the presence of increasing amounts of either anti-moesin or anti-CD3
We found that the binding of $^{51}$Cr-labeled T cells to the immobilized EFLNRWIT was abrogated by the anti-moesin mAb clone 38/87 in a dose-dependent manner, but not by anti-CD3 mAb (Fig. 5A), indicating that the major IL-2 peptide-binding moiety on T cells is indeed a moesin-like molecule.

Next, the binding of $^{125}$I-labeled EFLNRWIT-AY peptide to immobilized recombinant human moesin was tested in the absence or presence of increasing amounts of different T cell-specific mAbs, including anti-moesin mAb. As shown in Fig. 5B, in the absence of mAb, apparently a considerable amount of the labeled peptide was found to be bound to the recombinant immobilized moesin. The binding of $^{125}$I-labeled EFLNRWIT-AY was not affected by increasing amounts of anti-CD3, -CD69, or -VLA5 mAb. However, the moesin-specific mAb, clone 38/87, inhibited, in a dose-dependent manner, the binding of the labeled EFLNRWIT peptide to its immobilized ligand; a complete inhibition occurred at 1 $\mu$g/ml.

Finally, we examined the binding of radiolabeled EFLNRWIT-AY to immobilized recombinant moesin in the presence of increasing amounts of soluble recombinant moesin as a competitor. The results, shown in Fig. 5C, indicate that the recombinant soluble moesin, but not the control molecule KLH, inhibited, in a dose-dependent manner, the binding of the labeled EFLNRWIT to the immobilized ERM molecule. Taken together, these studies strongly suggest that the EFLNRWIT peptide interacts with moesin (or a closely related molecule) that appears to be expressed on the membranes of human T cells.

Inhibition of EFLNRWIT-induced T cell adhesion to FN by soluble recombinant moesin

Because we demonstrated that the IL-2 peptide EFLNRWIT regulates T cell adhesion to vessel wall and tissue components by interacting with a T cell surface-expressed moesin-like moiety, the involvement of moesin in the EFLNRWIT-induced T cell adhesion to FN was investigated. We assumed that the presence of soluble moesin in the T cell adhesion assay should interfere with IL-2 peptide-induced cell adhesion to FN by interacting directly with...
the peptide, and thus block its interactions with membranal moesin.

Hence, adhesion to FN of T cells pretreated with adhesion-inducing amounts of intact IL-2 or EFLNRWIT was examined by using increasing concentrations of recombinant moesin. The results indicate that T cell adhesion to FN induced by EFLNRWIT, but not by intact IL-2, was blocked, in a dose-dependent manner, by recombinant human moesin (Fig. 6). Recombinant soluble human moesin did not affect T cell adhesion to the identical, immobilized moesin (data not shown). This suggests that moesin binds the IL-2 peptide (but not the intact IL-2 molecule from which it is derived), and thereby, interferes with its adhesion-promoting abilities. Alternatively, the IL-2 peptide may bind to an undefined moiety other than moesin itself, and this binding may be interrupted by the binding of the anti-moesin mAb to T cells.

**FIGURE 5.** Analysis of the binding of EFLNRWIT to Jurkat cells and recombinant (rec.) moesin and the inhibition of this binding by anti-moesin mAb. A, Labeled Jurkat cells were incubated (4 h, 4°C) in microtiter wells precoated with the KLH-conjugated EFLNRWIT (0.25 μg/well) in the presence of increasing amounts of anti-moesin mAb, clone 38/87, and a control, anti-CD3 mAb. The plates were washed, the adherent cells lysed, and the radioactivity in the lysates determined. Radioactivity due to T cell binding to KLH-coated wells was subtracted. One experiment representative of three. B and C, Recombinant human moesin (18.5 ng/well) was applied (18 h, 4°C) onto microtiter well plates. The unbound protein was removed, and the wells were blocked by BSA (0.1% in PBS) before the binding (90 min, 4°C) of 125I-labeled EFLNRWIT-AY peptide (40 nM). The binding was inhibited by mAb against moesin, CD3, CD69, or CD49e (B), or soluble recombinant moesin or KLH (C). One experiment representative of three.

**FIGURE 6.** Recombinant (rec.) moesin inhibits the EFLNRWIT-induced T cell adhesion to FN. Human T cells were radiolabeled and then exposed to the EFLNRWIT peptide (1 fg/ml) or intact IL-2 (10 U/ml) that were preincubated (37°C, 4 h) with the indicated increasing amounts of soluble recombinant moesin, before being added to the wells. T cell adhesion (1 h) to immobilized FN was analyzed as described in Fig. 1. One experiment representative of four.

### Involvement of moesin in modulating the adhesion of activated T cells to FN and HA

Because the foregoing experimental findings indicate that a moesin-like molecule is involved in IL-2 peptide-mediated T cell adhesion, we further investigated whether the same anti-moesin mAb also interferes with the induction, by various proinflammatory mediators (that interact with nonmoesin moieties on T cells), of T cell adhesion to FN and HA. For this purpose, T cells were activated by various physiological and nonphysiological stimuli, then treated with anti-moesin mAb, and seeded onto the ligand-coated plates. Surprisingly, we found that the adhesion of T cells activated with the EFLNRWIT peptide, IL-2, as well as CD3 cross-linking by the anti-CD3 mAb, PMA, and the proadhesive chemokine MIP-1β, to FN and HA, was significantly abrogated by the anti-moesin mAb, clone 38/87, but not by the anti-LFA-1 mAb (Table I). Note that mAb anti-CD29 and -CD44 specifically inhibited T cell adhesion to FN or HA, respectively. Interestingly, the same mAb anti-moesin did not inhibit the binding of PMA-activated human monocytes (of the MonoMax-6 cell line) to FN. However, these cells did not express moesin on their surface and they did not respond to the EFLNRWIT peptide (data not shown).

### Discussion

The accuracy, effectiveness, and time-limited characteristics of the inflammatory reaction are facilitated by the rapid recognition of the inflamed context by tissue-specific receptors on immunocytes. Molecular interactions with such receptors, which influence the activation, adhesion, and migration of immune cells, are affected by inflammatory-regulating signals (21). These signals can be generated by (and their biological nature determined by) products of the migrating leukocytes themselves (e.g., cytokines, chemokines, growth factors, and enzymes) from the ECM (e.g., intact, degraded immobilized, or degraded soluble components), or, as shown here, products of the enzymatic degradation of inflammatory cytokines. During migration, leukocytes, especially T cells, can encounter, either sequentially or simultaneously, several of these soluble or immobilized modulators (3, 21–23), whereupon the chemical complexity of these signals is increased. Recently, we isolated, sequenced, and synthesized peptides resulting from the degradation of IL-2 by human neutrophil elastase (4). In this study, a role for one of these degradation products of inflammation and for the receptor that recognizes it in mediating the behavior of T cells in...
inflamed areas was demonstrated. We showed that 1) the effects (pro- or anti-adhesive) of the IL-2 peptide EFLNRWIT depend on its concentration and on the presence or absence of other signals and 2) the peptide exerts its functions by interacting with moesin, which is involved in cell adhesion.

First, we showed that even at concentrations as low as few fg/ml, the IL-2 peptide induced the adhesion of human T cells to FN, LN, CO, HA, VCAM-1, and ICAM-1. Interestingly, the dose-response curve of peptide-induced adhesion to FN was different from that of the intact IL-2-induced adhesion. In addition, the adhesion pattern of T cells induced by the elastase-generated IL-2 peptide is similar to that induced by certain cytokines and chemokines (22), which induce the adhesion of T cells by regulating their shapes and the avidities of adhesion receptors on their surfaces (3, 11). The movement of leukocytes depends on local concentrations of different chemotactic mediators; when the concentration of migratory mediators, such as IL-1β, IL-2, or IL-8, surpasses that of their maximal effective dose, the migration of lymphocytes and neutrophils is aborted (23, 24). This phenomenon may be due to the loss of a gradient of a single chemokine or a complex and opposing effect of two proadhesive or promigratory signals operating together. Future studies should aim to better understand the mechanism of the context- and concentration-dependent inhibition of lymphocyte adhesion by the EFLNRWIT peptide and to investigate whether this inhibition is due to interventions in the adhesive process by receptor desensitization (3). Recently, fMLP was observed to inhibit leukocyte migration induced by the subsequent exposure of the cells to either IL-8 or leukotriene B4 (22). Opiates, which induce chemotaxis of monocytes, inhibit cell migration induced by chemokines (25). Substance P, which by itself does not induce T cell adhesion to ECM, inhibits RANTES- and MIP-1β-induced T cell adhesion to FN (26).

HA, a glycosaminoglycan expressed on cell surfaces and complexed within the ECM, has been implicated in leukocyte migration; CD44-HA interactions are instrumental in recruiting T cells to inflammatory locations (6, 27). Therefore, we studied whether the IL-2 peptide also induces T cell adhesion to HA and the dose dependence and kinetics of this interaction. Although the IL-2 peptide induced T cell adhesion to immobilized HA, the kinetics of T cell adhesion to HA were different from those of T cell interactions with ECM glycoproteins, which are mediated by β1 integrins. More specifically, the T cell adhesion to FN occurred earlier than the adhesion to HA, and then decreased to background levels while appreciable adhesion to HA was detected. Moreover, the dose dependence of T cell adhesion to HA, induced by EFLNRWIT, showed an increasing-with-dose type of kinetic curve; its effect on T cell adhesion to FN showed a bell-shaped curve. These different patterns suggest that during migration to inflamed areas, T cells probably initially encounter low amounts of the IL-2 peptide, which induce adhesion and migration. However, as the T cells get closer to the inflammatory loci, they probably encounter increasingly higher concentrations of the mediator, and while their integrins are first turned off, the mediation of CD44-HA movement of the T cells is affected. These separate adhesive responses can be combined to ensure continuous and prolonged adhesive interaction of T cells with the ECM.

Regulation (induction or inhibition) of T cell adhesion (and probably also of migration) induced by EFLNRWIT appears to depend on the context in which the migrating T cells encounter this peptide. The peptide manifests its effect rapidly and functions at relatively low concentrations, which suggests that an IL-2 peptide-specific receptor is present on the T cells. Indeed, we found that two IL-2 peptide-specific receptors appear to be present on the T cells (Figs. 2 and 3), one of which is moesin, a protein that is intracellularly and extracellularly associated with the membrane of various cells. Here, moesin is implicated in the regulation of T cell adhesion induced by various physiological and nonphysiological activators (Fig. 6). Thus, moesin on T cells appears to be involved in dynamic changes in the function of T cells that occur during cell-substratum attachment and movement.

Moesin, a 78-kDa protein, has been characterized as being one of the ERM family of proteins (along with ezrin and radixin) involved in cell adhesion and membrane dynamics, probably because of their ability to link plasma membrane components with the actin cytoskeleton (7, 8, 15, 28). Although the functions of ERM proteins have not yet been fully delineated, these proteins are known to be key effector molecules of the downstream signal transduction pathways that modulate the plasticity of the membranes of cells (15, 29). In addition to its structural role as a cellular stabilizer, moesin was recently shown to interact, via its N-terminal domains, with cell surface-expressed adhesion receptors (CD44 and ICAM-3) on mobile (T cells) and nonmobile (fibroblast) cells (16, 30–32). Hence, moesin is involved not only in the formation of uropods, microvilli, and ruffling membranes, but also in establishing firm adhesive contacts between cells. By interacting with the actin filaments and certain signaling molecules associated with plasma membranes, such as Rho-associated kinases, moesin and related proteins have indeed been implicated in the structural and functional responses of different cells (19).

Accordingly, we suggest the existence of cell surface-expressed moesin on the freshly isolated, human PBL. Previously, the cellular localization of moesin was examined in lymphoblasts in which moesin was detected in the intracellular but not in the extracellular compartment (16). We have found that the expression of moesin on resting PBL (but not on human T cell lines; data not shown) can be up-regulated by activation (for 48 h) of the cells, not

Table I. Percentage of inhibition of T cell adhesion to FN and HA

<table>
<thead>
<tr>
<th>Activator</th>
<th>FN in the presence of mAb anti-</th>
<th>HA in the presence of mAb anti-</th>
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<tbody>
<tr>
<td></td>
<td>Moesin</td>
<td>LFA-1</td>
</tr>
<tr>
<td>EFLNRWIT</td>
<td>75 ± 5</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>IL-2</td>
<td>82 ± 5</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>mAb anti-CD3</td>
<td>78 ± 5</td>
<td>2 ± 2</td>
</tr>
<tr>
<td>PMA</td>
<td>72 ± 6</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>MIP-1β</td>
<td>70 ± 4</td>
<td>2 ± 2</td>
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</table>

* Labeled human T cells were activated with EFLNRWIT (1 fg/ml or 1 pg/ml for FN and HA, respectively), IL-2 (10 U/ml), MIP-1β, PMA (50 ng/ml), or anti-CD3 mAb (1 μg/ml) and seeded onto FN- or HA-coated wells in the presence of mAb anti-moesin (clone 38/87), anti-LFA-1, anti-CD29, and anti-CD44. T cell adhesion was determined after 1 h. One experiment representative of five.
only by PHA and PMA, but also by calcineurin A, a specific inhibitor of phosphatase 1 and 2A, which was shown to induce moesin phosphorylation (20). However, the expression level of moesin on activated lymphocytes was reduced after 5 days of PHA activation (data not shown). Our results imply that moesin, or a moesin-like moiety, is capable of acting as a cell surface receptor.

Recently, other research groups also reported the existence of moesin on macrophages, lymphocytes, fibroblasts, endothelial, and epithelial cells, and examined both the ligand-binding specificity and putative signal transduction capacities of moesin (17, 33–35). A similar phenomenon was observed with the cytoplasmic protein annexin II, which is expressed on the surface of various cell types (36). Interestingly, cell surface–expressed moesin interacts with heparan sulfate, LPS, and components of raftes and mesai vesicles (8, 18, 19, 28, 37). If moesin is indeed also a cell surface receptor, this implies that moesin, whether an intracellular or extracellular moiety, is a permissive receptor with a wide ligand-recognition specificity. Interestingly, a recent report demonstrates that moesin possesses a chemically composed N-terminal FERM domain, which is often present in cell signaling and cytoskeletal proteins. Upon binding other peptides and/or phospholipid ligands, this moiety could provide varying levels of activation (38).

This study suggests that during inflammatory reactions, the products of enzymatic degradation of proinflammatory mediators can regulate the behavior of immune cells. Additional studies are needed to elucidate the mechanisms underlying the regulation of immune cell adhesion by moesin (and IL-2 peptides). One such research direction may be to clarify the functional association of moesin and T cell adhesion receptors. Our preliminary findings indicate that moesin, upon responding to the IL-2 peptides, associates physically with surface β1 integrins (data not shown), thereby up-regulating their ligand-binding functions.

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References
