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Coengagement of ICAM-3 and Fc Receptors Induces Chemokine Secretion and Spreading by Myeloid Leukocytes

Julie M. Kessel,* Joel Hayflick,** Andrew S. Weyrich,* Patricia A. Hoffman,** Michael Gallatin,** Thomas M. McIntyre,**§ Stephen M. Prescott,**§ and Guy A. Zimmerman²*§

ICAM-3 is expressed at high levels on myeloid leukocytes, but its function on these cells is unknown. We tested the hypothesis that it transduces outside-in proinflammatory signals using immobilized mAbs to engage ICAM-3 on freshly isolated human monocytes and neutrophils. Two immobilized Abs that recognize epitopes in the extracellular domain 1 of ICAM-3, which is critical for recognition by the $\alpha_{4}/\beta_{2}$ integrin, potently induced secretion of MIP-1α, IL-8, and MCP-1 by monocytes and triggered IL-8 secretion by neutrophils. These chemokines are products of immediate-early genes that are induced when myeloid cells are activated. Chemokine secretion induced by “triggering” Abs was greater than that induced by isotype-matched immobilized Abs against ICAM-1, ICAM-2, PECAM-1, control Igs, or immobilized control proteins. Coengagement of ICAM-3 and Fc receptors (FcγRI or FcγRII) was required for maximal chemokine secretion by monocytes. Microscopy documented that there is also dramatic spreading of monocytes when surface ICAM-3 is engaged by immobilized Abs. Spreading was induced by Fab and $F(ab')_{2}$ fragments of triggering anti-ICAM-3 mAb, demonstrating direct outside-in signaling, but was not required for chemokine secretion. These experiments indicate that ICAM-3 may transmit outside-in signals when it is engaged by $\beta_2$ integrins during myeloid cell-cell interactions in inflammatory lesions. Binding of Fc receptors by Ig in the local environment can amplify the responses. The Journal of Immunology, 1998, 160: 5579–5587.

Interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) activate transcription factors and provoke diverse cellular functions (1). These proinflammatory cytokines are produced by a variety of cell types in response to tissue injury and infection (1). IL-1 and TNF-α are also released from activated leukocytes and serve as chemokines that attract neutrophils and monocytes to sites of infection. Both of these cytokines activate intracellular signaling cascades (2). Here we show that engagement of extracellular domains of ICAM-3 on freshly isolated human monocytes induces activation of intracellular signaling cascades, a process that is termed outside-in signaling and that causes altered cellular function and responses. Outside-in signaling mediates juxtacrine activation during adhesive interactions involving leukocytes and other cells (2). We show that engagement of extracellular domains of ICAM-3 on freshly isolated human monocytes and neutrophils (PMNs) by mAbs induces synthesis and secretion of chemokines and causes cellular spreading. Thus, ICAM-3 may be a juxtacrine signaling ligand on myeloid cells when it binds $\beta_2$ integrins on other leukocytes in inflamed vessels and tissues.

Materials and Methods

**Reagents**

HBSS and medium 199 (M199) (with EBSS, t-glutamine and HEPES) were from BioWhittaker (Walkersville, MD). Human serum albumin (25%) was from Baxter Healthcare (Glendale, CA). Polymyxin B sulfate, fatty acid-free BSA, LPS (Escherichia coli serotype 0111:B4), herbimycin A (from Streptomyces hygroscopicus, in DMSO), and cytochalasin D

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Radioactivity was measured using a Beckman Gamma 5500 counter (Torrance, CA).

**Assays of leukocyte spreading**

Plastic chamber slides (Nunc) were incubated overnight with 200 to 300 μl/well of HBSS alone or HBSS with 10 μg/ml of Ab. Unbound sites were blocked by incubating each well with 500 μl of 2% serum albumin for 2 h at room temperature. The plates were washed with 0.1% Tween-20 in HBSS three times and then with HBSS twice. Monocytes (300–450 μl, 0.25–0.5 × 10⁶ cells/ml) in serum-free M199 were added under sterile conditions. Control buffer (HBSS containing 2% human albumin) or LPS in buffer was added in 50-μl aliquots to some of the wells in some experiments. The cells were incubated for the designated time period at 37°C in 5% CO₂/95% air. The wells were then washed with HBSS and adherent cells were fixed and stained using a Diff-Quick (Baxter McGaw Park, IL) staining kit. Fixed cells were scored as rounded (no spreading) or spread using morphologic characteristics previously illustrated (18). Random fields were examined under ×40 magnification until 100 cells were characterized. Observations of live cells by inverse light microscopy were also made during incubations in experiments measuring chemokine secretion.

**ELISAs**

ELISAs for chemokines and cytokines were done as described (18) or using minor modifications of this method. Briefly, Costar (Cambridge, MA) 96-well E.I.A. plates were coated with anti-human mAbs (4 μg/ml) against MIP-1α, IL-8, MCP-1, or TNF-α. After an overnight incubation at 4°C, the plates were blocked with 2% BSA in PBS, and then washed four times with PBS/0.05% Tween-20. Standards and unknown samples were added in 50-μl aliquots (neat, or diluted 1:2 to 1:10 with PBS/BSA) and incubated for 90 min for IL-8 and TNF-α or 60 min for MIP-1α and MCP-1. Biotinylated anti-human mAbs against the respective chemokines were added and incubated for 90 min for IL-8 and TNF-α and 45 min for MCP-1 or MIP-1α. In all, 50 μl/well of avidin-peroxidase (8 μg/ml) were added and incubated for 30 min for assays of IL-8 or TNF-α and 60 min for assays of MCP-1 or MIP-1α. Plates were developed with 100 μl/well of peroxidase substrate. The reaction was stopped with 50 μl/well of 1N H₂SO₄ and the OD was measured at 492 nm.

**Results**

**Engagement of ICAM-3 induces chemokine secretion by monocytes**

To test the hypothesis that engagement of ICAM-3 on myeloid leukocytes mediates outside-in signaling, we immobilized Abs against ICAM-3 and determined whether their binding to monocytes triggered the synthesis and secretion of chemokines and cytokines. In parallel studies, engagement of ICAM-3 on monocytes induced transcripts for IL-8 and MIP-1α and engagement of ICAM-3 on a human myeloid cell line via immobilized Ab induced rapid sustained up-regulation of messenger RNA for MIP-1α and reporter gene activity linked to MIP-1α promoter elements (J. Kessel, unpublished observations; J. Hayflick et al., unpublished observations). In the current experiments, however, we focused on secretion of MIP-1α and other chemokines as the
biologically relevant “readout” since there is evidence that adhesion-dependent signaling can induce mRNAs without synthesis of the corresponding proteins by monocytes under some circumstances (19). Secretion of MIP-1a was potently induced when ICAM-3 was engaged by immobilized mAb 8.1 and mAb 5.1 (Table I) but was at basal levels when monocytes were incubated with a control protein, albumin (Figs. 1 and 2). Secretion of IL-8 was also induced (Figs. 1 and 2). MIP-1α and IL-8 secretion triggered by mAb 5.1 or 8.1 was greater than that by monocytes incubated with irrelevant Igs or Abs to other surface determinants (Figs. 1 and 3, and Table I; also see below). In an additional experiment, Abs 8.1 and 5.1 also triggered MCP-1 secretion (950 pg/ml MCP-1 induced by mAb 8.1, 1000 pg/ml induced by mAb 5.1, 200 pg/ml induced by immobilized albumin at 8 h of incubation).

mAbs 8.1 and 5.1 are directed against the first N-terminal extracellular domain of ICAM-3 (domain 1) (16), a region that is critical for interaction with its natural ligand, αLβ2 integrin (6, 16, 20, 21). Several other immobilized anti-ICAM-3 Abs studied in parallel were much less potent as inducers of chemokine secretion, including some Igs directed against domain 1 determinants (Table I, Fig. 2) (16, 22). This indicates that particular structural features

within domain 1 influence outside-in signaling when ICAM-3 is engaged. Binding of radiolabeled monocytes to immobilized anti-ICAM-3 Abs with different triggering potencies was equivalent, although there was some variation from experiment to experiment (not shown).

Engagement of ICAM-3 by mAb 5.1 or 8.1 induced time-dependent secretion of chemokines by monocytes. Release of MIP-1α and IL-8 was below the thresholds of detection for the assays immediately following isolation of the leukocytes, but rose rapidly between 2 and 8 h after ICAM-3 was engaged and continued at 18 to 24 h (Fig. 3 and data not shown). Monocytes examined at 2 to 24 h of serum-free incubation were viable, as assessed by trypan blue exclusion.

To determine the functional significance of chemokine secretion triggered by outside-in signaling via ICAM-3, we incubated monocytes with immobilized mAb 8.1 for 8 h, a time point that corresponded to near-maximal stimulation (Fig. 3), and asked whether the conditioned supernatants contained activity that induced PMN chemotaxis. The conditioned supernatants stimulated PMN transmigration across 5-μM filters; the magnitude of stimulated migration was threefold greater than that induced by conditioned supernatants from monocytes incubated on immobilized albumin in parallel, and approached that stimulated by the chemoattractant FMLP (not shown). Transmigration of PMNs across endothelial monolayers grown on filters was also induced. The presence of chemotactic activity in conditioned supernatants from monocytes incubated with immobilized mAb 8.1 is consistent with secretion

FIGURE 1. Engagement of ICAM-3 by a “triggering” mAb against ICAM-3 induces MIP-1α and IL-8 secretion by human monocytes. Immobilized Ab 8.1 was used to engage ICAM-3 on human monocytes for 8 h as described in Materials and Methods, and cell-free supernatants were analyzed by ELISA for MIP-1α (A) and IL-8 (B). The number of experiments and the statistical significance comparing the release of monocytes incubated with immobilized anti-ICAM-3 vs immobilized irrelevant IgG are shown in each panel. The p value represents a two-tailed, paired t test comparing chemokine secretion by monocytes incubated with immobilized mAb 8.1 vs IgG isotype control. The thresholds of detection of chemokines were 0.2 ng/ml for IL-8 and 0.1 ng/ml to 0.15 ng/ml for MIP-1α in these assays.

FIGURE 2. Anti-ICAM-3 Abs differ in potency as triggers for MIP-1α and IL-8 secretion. mAbs 1.1, 2.1, 8.1, and 5.1 were used to engage domain 1 of ICAM-3 on human monocytes, and secretion of chemokines was measured as described in Materials and Methods and Figure 1. mAbs 8.1 and 2.1 are of the IgG1 isotype and mAbs 5.1 and 1.1 are IgG2a; each Ab recognizes domain 1 of ICAM-3 (Table I).
We found that PMNs release IL-8 when ICAM-3 is engaged by ICAM-3 mAb as triggers for chemokine secretion were similar in PMNs, ICAM-3 is more highly expressed than ICAM-1, and ICAM-2 is not detectable (not shown). These patterns are similar to those in previous experiments using different Abs (4, 6). We then compared mAb 8.1 and Abs of the same isotype directed against ICAM-1 and ICAM-2 as inducers of outside-in signaling in monocytes. The rank order of potency of these immobilized Abs as triggers for chemokine secretion was anti-ICAM-3 >> anti-ICAM-2 >> anti-ICAM-1 (Fig. 4). In additional experiments, we compared engagement of ICAM-3 to ligation of another member of the IgG superfamily that is expressed on monocytes and that mediates outside-in signaling in leukocytes, PECAM-1 (25–27; reviewed in Ref. 28). An immobilized Ab against PECAM-1, hec-7 (IgG2a, directed against the first N-terminal extracellular domain; Refs. 29, 30), triggered no more secretion of chemokines than did immobilized albumin or anti-ICAM-1 studied in parallel, whereas immobilized mAbs 5.1 and 8.1 induced release of MIP-1α and IL-8, as expected from previous experiments (Fig. 5). When examined by flow cytometric analysis, hec-7 bound only slightly less well to monocytes than did the Abs against ICAM-3 (not shown), indicating that PECAM-1 and ICAM-3 have roughly equivalent representation on the monocyte surface.

**Engagement of ICAM-3 induces chemokine secretion by PMNs**

We found that PMNs release IL-8 when ICAM-3 is engaged by triggering anti-ICAM-3 Abs (Fig. 6). In contrast to the result with monocytes, no MIP-1α or MCP-1 secretion was induced. Again, no TNF-α secretion was triggered when PMNs were incubated with activating anti-ICAM-3 Abs. The relative potencies of anti-ICAM-3 mAb as triggers for chemokine secretion were similar in PMNs and monocytes (Figs. 1, 2, and 6), although this was not examined as extensively in PMNs as in the latter cell type.

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**FIGURE 3.** Engagement of ICAM-3 on human monocytes induces time-dependent secretion of chemokines. Freshly isolated monocytes were incubated with immobilized anti-ICAM-3 (mAb 8.1) (circles), immobilized irrelevant IgG (squares), or immobilized albumin (cross marks) for the times shown. Supernatants were collected and assayed for MIP-1α (A) or IL-8 (B) as described in Materials and Methods. Similar time courses were seen in a second experiment performed under similar conditions.

**FIGURE 4.** ICAM-3 mediates potent outside-in signaling of chemokine secretion by monocytes when compared with ICAM-1 and ICAM-2. Immobilized Abs were used to engage ICAM family members on human monocytes for 8 h, and chemokine secretion was analyzed by ELISA as described in Figure 1 and Materials and Methods. The mAbs used were: mAb 8.1 for ICAM-3, mAb 18E3D for ICAM-1, and mAb H11A for ICAM-2. Each mAb is of the IgG1 isotype. Secretion of MIP-1α by monocytes incubated with immobilized albumin and with immobilized irrelevant IgG1 (“isotype control”) was measured in parallel. The p value for a two-tailed, paired t test comparing chemokine secretion by monocytes incubated with immobilized anti-ICAM-3 mAb vs IgG isotype control is shown.
Coengagement of ICAM-3 and Fc receptors triggers enhanced chemokine secretion by human monocytes

We analyzed chemokine secretion by monocytes incubated with full-length anti-ICAM-3 mAbs, with F(ab\(^{-}\))\(_2\) or Fab fragments of the same Abs, or with irrelevant murine IgG1 or IgG2a to examine the interplay between Fc receptors and ICAM-3. We first examined coengagement involving Fc\(\gamma\)RII, which recognizes murine IgG1 (reviewed in Ref. 31), using mAb 8.1 (IgG1 class). Separate engagement of ICAM-3 and Fc\(\gamma\)RII triggered a low level of or no chemokine secretion. This was shown in experiments in which a F(ab\(^{-}\))\(_2\) fragment of mAb 8.1 or full-length murine IgG1 was immobilized separately, and chemokine secretion by adherent monocytes was compared with that of monocytes incubated on full-length mAb 8.1 (Fig. 7). In a second strategy, we used a blocking Ab against Fc\(\gamma\)RII, mAb IV.3. Preincubation of monocytes with Fab or full-length IV.3 reduced IL-8 secretion by \(\sim 85\%\) when ICAM-3 was engaged by mAb 8.1 as the complete Ig (Fig. 8).

In additional experiments, engagement of Fc\(\gamma\)RI alone with immobilized murine IgG2a, or ICAM-3 alone with the F(ab\(^{-}\))\(_2\) fragment of mAb 5.1, triggered submaximal MIP-1\(\alpha\) secretion when compared with full-length mAb 5.1 (not shown). mAb 5.1 is of the IgG2a isotype, which is recognized by Fc\(\gamma\)RII (31). As previously reported (32), nonimmune full-length murine IgG2a alone induced IL-8 secretion. However, the levels of chemokine release were lower than those induced by mAb 5.1. Ligation of Fc\(\gamma\)RI alone with murine IgG2a also induced MIP-1\(\alpha\) secretion but, again, at levels lower than those observed with coengagement of ICAM-3. In some experiments, an IgG2a mAb directed against a different surface adhesion molecule (PECAM-1) did not induce chemokine secretion above that of monocytes incubated on albumin, whereas mAb 5.1 triggered release (Fig. 5).

Engagement of ICAM-3 induces monocyte spreading

Cellular spreading is linked to induction of genes in a variety of cell types (33, 34) and may be important in the mechanism of expression of chemokine genes in monocytes (35–37). We next examined the relationship of cellular spreading to chemokine secretion when ICAM-3 is engaged on monocytes. We found that there was dramatic spreading of monocytes on immobilized mAb 5.1, triggered submaximal MIP-1\(\alpha\) and IL-8 secretion when compared with full-length mAb 5.1 (not shown). mAb 5.1 is of the IgG2a isotype, which is recognized by Fc\(\gamma\)RI (31). As previously reported (32), nonimmune full-length murine IgG2a alone induced IL-8 secretion. However, the levels of chemokine release were lower than those induced by mAb 5.1. Ligation of Fc\(\gamma\)RI alone with murine IgG2a also induced MIP-1\(\alpha\) secretion but, again, at levels lower than those observed with coengagement of ICAM-3. In some experiments, an IgG2a mAb directed against a different surface adhesion molecule (PECAM-1) did not induce chemokine secretion above that of monocytes incubated on albumin, whereas mAb 5.1 triggered release (Fig. 5).
hibit secretion of MIP-1α by cytochalasin D, which inhibits actin polymerization for chemokine secretion. Monocytes were preincubated with the ICAM-3, we examined the requirement for cytoskeletal alteration compared with cells with ICAM-3 coengaged (Fig. 9).

Because cellular spreading was induced by engagement of ICAM-3, we examined the requirement for cytoskeletal alteration for chemokine secretion. Monocytes were preincubated with the fungal product cytochalasin D, which inhibits actin polymerization (reviewed in Ref. 38). In concentrations sufficient to inhibit monocytic cell spreading, cytochalasin D did not consistently inhibit secretion of MIP-1α or IL-8 induced by triggering mAbs 8.1 or 5.1 (n = 5). Thus, spreading and chemokine secretion could be dissociated. This result is consistent with a previous experiment in which an immobilized Ab that is weak as a “trigger” for chemokine secretion by myeloid leukocytes, mAb 1.1 (Table I, Figs. 2 and 6), induced dramatic spreading but no chemokine release (18).

Discussion

At the report of its molecular cloning, ICAM-3 was proposed to be an adhesion molecule that is particularly important in initiation of immune responses, in part because of its higher level of constitutive expression on lymphocytes compared with ICAM-1 and ICAM-2 and also because of characteristics of its interaction with α4β1 integrin (4–6, 39). Several studies indicate that ICAM-3 can tether and mediate outside-in signaling of lymphocytes, lymphocytic cell lines, and thymocytes (39–45). However, ICAM-3 is also expressed at high levels on myeloid leukocytes of adults and newborn infants (4–6, 39) (J. Kessel, manuscript in preparation). This indicates that it may have particular importance in tethering and signaling of these cells in addition to its putative roles in lymphocyte-mediated immune responses. In studies of adhesion, we found that ICAM-3 can tether myeloid cells (J. Kessel et al., unpublished observations). Here we show that engagement of ICAM-3 on monocytes and PMNs by specific immobilized mAbs induces secretion of chemokines and cellular spreading. We acknowledge that engagement by mAbs does not absolutely mimic responses induced by engagement by natural ligands, either quantitatively or qualitatively. Nevertheless, this strategy has been useful in defining the signaling potential of adhesion molecules and for identifying structural features that contribute to this function (22, 27, 30, 46–51). Our experiments using this approach indicate that ICAM-3 can transmit outside-in signals in monocytes and PMNs and suggest that it serves as a juxtacrine ligand (2, 52) when they interact with other leukocytes.

Immobilized Abs against ICAM-3 triggered secretion of MIP-1α, IL-8, and MCP-1 by monocytes (text and Figs. 1–5), cells known to produce and release chemokines in an adhesion-dependent fashion (18). We also showed that engagement of ICAM-3 on freshly isolated PMNs induces IL-8 secretion (Fig. 6). Synthesis and secretion of chemokines is a recently identified activation response of PMNs, which previously were thought to be incapable of new synthesis of inflammatory proteins (53). In contrast to this...
result, ICAM-3 was reported to be an inhibitor of PMN activation when adhesion to endothelial cells was assayed as the functional response (54). While a possible explanation for the latter observation is that ICAM-3 induces “negative” signaling (55, 56) of adhesion in PMNs, in other experiments we found that some of the same Abs that trigger chemokine release (Table I; Fig. 6) also induce PMN aggregation mediated by $\beta_2$ integrin activation (M. Feldhaus, J. Kessel et al., manuscript in preparation). Thus, our findings are consistent with ICAM-3 as a positive modulator of myeloid leukocyte function rather than as an inhibitor.

The Abs that most potently induced chemokine secretion by human monocytes and PMNs are directed against epitopes in the first N-terminal extracellular domain (domain 1) of ICAM-3 (6, 16) (Table I). A domain 1 Ab also directly triggered spreading of monocytes (Fig. 9). Domains 1 and 2 of ICAM-3 are involved in its recognition by $\alpha_\delta/\beta_2$ integrin (5, 16, 20, 57). Thus, binding of the $\alpha_\delta/\beta_2$ integrin to this region on ICAM-3 may trigger outside-in signaling and chemokine secretion during PMN and monocyte aggregation or in other leukocyte-leukocyte interactions involving these cells. Lymphocytes are also activated by Abs directed against domain 1 of ICAM-3 (20, 22, 40, 41, 51, 58, 59), supporting the possibility that this region of the molecule is important in signaling. The variable potency of Abs that bind to epitopes in domain 1 as triggers for chemokine secretion by monocytes (Table I) indicates that specific structural features are involved in outside-in signaling via ICAM-3. While it is possible that differences in affinity of the Abs accounted for their differences in potency as triggers, this seems unlikely under the conditions of our experiments, since there was equivalent binding of radiolabeled monocytes to the immobilized mAb (see Results).

ICAM-1 and ICAM-2 are reported to have outside-in signaling properties under some conditions (60–65). We found that engagement of ICAM-3 by triggering mAb induced chemokine secretion by monocytes whereas isotype-matched Abs against ICAM-1 and ICAM-2 did not stimulate secretion greater than that by cells incubated with immobilized control IgG (Fig. 4). While one potential explanation for our findings is that ICAM-3 is more effective as an outside-in signaling molecule than are the other two family members, no conclusions can be drawn on this point because of the variable potency of the anti-ICAM-3 Abs and the small number of Abs against ICAM-1 and ICAM-2 that we examined (Table I). Alternatively, a second explanation is that the pattern is due to the relative densities of ICAM-3 vs ICAM-1 and ICAM-2 on the surface of monocytes, where ICAM-3 is more highly represented (see Results). However, we also found that engagement of PECAM-1, another IgG superfamily member that mediates outside-in signaling under some conditions (27, 28, 47), did not induce MIP-1$\alpha$ or IL-8 secretion. The binding of the Abs against ICAM-3 and PECAM-1 were similar by flow cytometry, suggesting that relative surface density is not the only critical variable.

The cytoplasmic pathways that mediate outside-in signaling of chemokine expression when ICAM-3 is engaged on myeloid leukocytes are not yet defined. As previously noted (see Results), many observations indicate that intracellular signals generated by cellular spreading and/or cytoskeletal reorganization are important in the expression of chemokines and other gene products under certain conditions. In addition, Abs against domain 1 of ICAM-3 trigger both cell spreading and cytokine and chemokine secretion in other leukocyte types; the latter events are dependent on specific regions of the cytoplasmic tail of ICAM-3, consistent with outside-in signaling (51). Thus, spreading and chemokine expression could be mechanistically linked in a sequential or amplifying fashion when ICAM-3 is engaged. We found, however, that cytochalasin D did not block chemokine secretion induced by engagement of ICAM-3 on monocytes even though it inhibited cellular spreading in parallel. This indicates that reorganization of the actin cytoskeleton (38, 66), and molecular events resulting from changes in shape secondary to the spreading response (33, 34), are not required for chemokine expression when ICAM-3 is engaged on this cell type, suggesting divergence of the intracellular pathways leading from the cytoplasmic tail to the two effector responses. In myeloid leukocytes, outside-in signaling of chemokine secretion via ICAM-3 may involve phosphorylation of serine residues in the cytoplasmic tail (51) followed by phosphorylation of intracellular proteins on tyrosine. Preliminary experiments indicate that tyrosine kinase inhibitors reduce chemokine secretion when ICAM-3 is engaged on freshly isolated human monocytes (J. Kessel et al., unpublished observations). Tyrosine kinase pathways also mediate signaling in lymphocytic cells when ICAM-3 is engaged (44). This likely occurs by direct or indirect interaction of ICAM-3 with intracellular “nonreceptor” tyrosine kinases, causing them to phosphorylate downstream targets since tyrosine residues in the cytoplasmic tail of ICAM-3 on activated leukocytes were not phosphorylated in our earlier studies (51), although others have reported a different result (67).

Our experiments demonstrated costimulation of chemokine secretion when ICAM-3 and Fc receptors on monocytes were engaged (Fig. 7, 8). Human monocytes constitutively express FcγRI (CD64) and FcγRII (CD32) (31, 68–70). Human FcγRI recognizes murine IgG2a, the isotype of triggering mAb 5.1, and FcγRII recognizes murine IgG1, the isotype of triggering mAb 8.1 (Table I) (reviewed in Ref. 31). It has been reported previously that engagement of FcγRI by Ig induces IL-8 release by monocytes (32). We confirmed this observation but also found that irrelevant IgG2a and “binding” anti-ICAM-3 mAb of this class did not induce the same magnitude of IL-8 or MIP-1$\alpha$ secretion as did mAb 5.1 (see Results, Table I, and Fig. 5). This indicates that activation through FcγRI by the Fc portion of mAb 5.1 was not the exclusive mechanism of outside-in signaling of chemokine secretion induced by this Ab and that engagement of ICAM-3 was required for the full response. In a second series of experiments, we found that engagement of ICAM-3 and FcγRII is required for triggering of maximal chemokine secretion by mAb 8.1 (Figs. 7 and 8). Together, the experiments indicate that costimulation of ICAM-3 and Fc receptors amplifies outside-in signaling and the secretion of MIP-1$\alpha$ and IL-8 by monocytes. Similarly, maximal and most rapid monocyte spreading was induced by intact triggering Abs, although Fab and F(ab′)$^2$ fragments of mAb 8.1 also induced spreading, demonstrating direct signaling by engagement of ICAM-3 (Fig. 9).

The requirement for coengagement of ICAM-3 and Fc receptors for maximal chemokine secretion and rapid spreading suggests that intracellular signaling pathways linked to these surface structures converge and are integrated (2, 18). Cytoplasmic domains in the FcγRI and FcγRII molecular complexes contain Ag receptor activation motifs (ARAMs), also termed immunoreceptor tyrosine-based activation motifs, which associate with Src protein tyrosine kinases (71, 72). A variety of cell surface molecules that mediate outside-in signaling bear these cytoplasmic sequences (56, 73). The cytoplasmic domain of ICAM-3 does not contain consensus ARAM motifs (J. Kessel and E. Trayer, unpublished observations). However, as noted above, preliminary experiments indicate that ICAM-3 on monocytes is linked to tyrosine kinase-dependent signaling; this transduction pathway may converge on pathways linked to ARAM-bearing Fc domains.

Because Fc receptors recognize monomeric and/or aggregated human IgG as well as murine IgG (31, 32, 71), coengagement of these structures and ICAM-3 on human monocytes or PMNs may
be important in syndromes of pathologic inflammation of the lungs, kidneys, or other organs in which immune complexes or Ig are deposited (74–76) and in which leukocyte-leukocyte interactions involving activated β₂ integrins and ICAM family members occur. Our experiments suggest that under these circumstances triggering of synthesis and secretion of chemokines, including MCP-1α and IL-8, which can further amplify inflammation by recruiting additional leukocytes (reviewed in Refs. 76 and 77), may be critical consequences of the signaling. In contrast, chemokine secretion is reduced or absent when coengagement of ICAM-3 and Fc receptors does not occur (Figs. 7 and 8), suggesting a control mechanism that limits proinflammatory signaling when they are individually ligated. These observations suggest that a major function of ICAM-3 may be as a costimulatory ligand. In T lymphocytes and APCs, ICAM-3 has costimulatory functions (22, 40, 78). Transfection of ICAM-3 into deficient Jurkat T lymphoblastoid cells complements a defect in IL-2 release when the TCR is engaged, documenting a costimulatory role (51). Furthermore, we have found that engagement of ICAM-3 amplifies monocyte responses when they are activated through plasma membrane signaling receptors (A. S. Weyrich, S. Davies, et al., unpublished observations). Such cooperative actions involving surface molecules appear to be key events in signal integration and in juxtaparan signaling systems in leukocytes and in a variety of other cell types (2, 18).

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References


