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*J Immunol* 1998; 161:3330-3339; ;
http://www.jimmunol.org/content/161/7/3330

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Roles of Chemokines and Receptor Polarization in NK-Target Cell Interactions

Marta Nieto,* Francisco Navarro,* Juan José Perez-Villar,* Miguel Angel del Pozo,* Roberto González-Amaro,* Mario Mellado,† José M. R. Frade,† Carlos Martínez-A,† Miguel López-Botet,* and Francisco Sánchez-Madrid2*

We report that the ability of NK cells to produce chemokines is increased in NK-target cell conjugates. The chemokines produced play a critical role in the polarization and recruitment of NK cells as well as in the NK effector-target cell conjugate formation. Chemokines induce the formation of two specialized regions in the NK cell: the advancing front or leading edge, where chemokine receptors CCR2 and CCR5 cluster, which might guide the cells toward the chemotactic source, and the uropod, where adhesion molecules ICAM-1 and -3 are redistributed. NK cell polarity was intrinsically involved in conjugate formation. The redistribution of both adhesion receptors and CCR was preserved during the formation of NK-target cell conjugates. Time-lapse videomicroscopy studies of the formation of effector-target conjugates showed that morphologic poles are also functionally distinct; while the binding to target cells was preferentially mediated through the leading edge, the uropod was found at the rear of migrating NK cells and recruited additional NK cells to the vicinity of K562 target cells. Inhibition of cell polarization and adhesion receptor redistribution blocked the formation of NK-K562 cell conjugates and the cytotoxic activity of NK cells. We discuss the implication of NK-cell polarization in the development of cytotoxic responses. The Journal of Immunology, 1998, 161: 3330–3339.

NK cells belong to a distinct lineage of lymphocytes capable of killing a variety of target cells, including tumor cells, cells infected by viruses or bacteria, and some normal cells (1). NK cells resemble CTL in certain respects, including the cytokine production profile and the use of a perforin-dependent lytic mechanism, but they do not rearrange TCR or Ig genes. The migration and recruitment of NK cells from blood vessels to target tissues are the first steps in the cascade of events in the NK cytotoxic response. The diverse factors involved in this complex process have recently been explored. NK cells exhibit rapid migration in vitro (2) and express a repertoire of adhesion molecules from β1 and β2 integrin families, enabling them to interact with endothelial cell counter-receptors (3, 4) and extracellular matrix proteins (5).

It has been widely proposed that chemokines are the major soluble mediators of leukocyte recruitment in inflammation and immunity (6–8). These molecules are differentially secreted by platelets and a broad spectrum of nucleated cells, including endothelial cells, T lymphocytes, and monocytes (7, 9, 10). Although little is known about the effects of chemokines on human NK cells, it has been shown that these cytokines induce chemotaxis and chemokinesis of NK cells in vitro. In addition, it has been reported that chemokines stimulate Ca2+ mobilization and cytolytic granule release, promote cytotoxic activity, and regulate the adheriveness of NK cells (11–14). NK cells, in turn, synthesize chemotactic factors, including IL-8, MIP-1α,β, and lymphotactin (15–17), which may induce the migration of additional effector cells into the target tissue. Chemokines specifically interact with receptors that possess seven transmembrane domains and are coupled to a G protein signaling pathway. Several of these receptors have been cloned and classified into two groups, CCR and CXCR (6, 18), but their patterns of expression on NK cells have not been fully elucidated.

Leukocyte migration requires cell polarization, a phenomenon that is involved in many other processes, such as cell differentiation, vectorial transport of molecules across cell layers, induction of immune response, cognate interactions between APC and T cells, and target cell recognition and killing (19–24). Cell polarization is required for the release of NK cytolytic granules as well as for the formation of conjugates between killer cells and their target cells (25). We recently reported that chemokines and other chemotactic cytokines induce in T cells the polarization of chemokine receptors (CCR) to the cell leading edge, probably directing the cells along the chemokine gradient (26). In addition, chemokines induce the formation of a cell projection at the rear of the cell, termed the uropod, accompanied by redistribution of specific adhesion molecules such as ICAM-1, ICAM-3, CD43, and CD44 to this structure (27–29).

We have studied the polarization of NK cells induced by chemokines or during NK-target cell interaction as well as the possible role of the cellular uropod in the migration and recruitment of NK cells. We have found that the leading edge of NK cells concentrates chemokine receptors and is implicated in target interactions, while the uropod mediates homotypic NK cell interaction, which might be important for the recruitment of these cells into

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1 This work was supported by Grant SAF 96/0039 from the Ministerio de Educación y Ciencia, Grant 07/44/96 from the Comunidad Autónoma de Madrid, a grant from Fundación Científica de la Asociación Española contra el Cancer (to F.S.-M.), and fellowships from the Fondo de Investigaciones Sanitarias BAE 97/5089 (to M.A.P.) and the Ministerio de Educación y Ciencia (to M.N.). The Department of Immunology and Oncology was founded and is supported by the Consejo Superior de Investigaciones Científicas and Farmacia and Upjohn.

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3 Abbreviations used in this paper: MIP-1, macrophage inflammatory protein-1; rh, recombinant human; MCP, monocyte chemoattractant protein; FN, fibronectin.
target tissues. We discuss the implication of these findings in relation to mechanisms relevant to the in vivo migration of NK cells.

Materials and Methods

Abs, cytokines, and reagents

The mAbs anti-ICAM-3 HP2/19 (IgG2a), anti-CD11a TP1/40, anti-CD45 D39 (IgG1), and anti-CD94 HP5B1 (IgG2a) have been previously described (30–32). The anti-ICAM-1 MEM 111 was a gift from Dr. V. Hooijtj (Institute of Molecular Genetics, Vidienska, Czech Republic), and the anti-CD16 B73.1 (IgG1) (33) was a gift from Dr. B. Perussia. The anti-CD56 mAb K218 (IgG1) was provided by Dr. A. Moretta (Istituto Nazionale per la Ricerca sul Cancro e Centro Biotecnologie Avanzate, University of Genova, Genova, Italy), and the L16 mAb was donated by Dr. C. Figdor (Nijmegen, The Netherlands). Anti-CCR2 (CCR2-03) and anti-CCR5 (CCR5-01) mAbs have previously been described (30–32). Recombinant human (rh) RANTES, MCP-1, MIP-1α, MIP-1β chemokines, and rhIL-15 were purchased from PeproTech (London, U.K.), and rhTNF-α (sp. act., 5 × 10^6 U/mg; purity, >95%) was provided by Genentech (San Francisco, CA). The rhIL-2 was provided by Hoffmann-La Roche (Nutley, NJ).

Protein substrates

Recombinant chimeric ICAM-1-Fc, consisting of the total extracellular domains of ICAM-1 fused to the IgG1 Fc fragment, was obtained as previously described (34). Briefly, COS-7 cells were transiently transfected with ICAM-1-Fc (ICAM-1 cDNA cloned in pcDNA3/IgG1). After 4 days, culture supernatants were precipitated with ammonium sulfate, and chimeric proteins were isolated using protein A coupled to Sepharose (Pharmacia, Uppsala, Sweden). The tryptic 38- and 80-kDa fibronectin fragments (Meron, CA) were gifts from Dr. A. Garcia-Pardo (Centro de Investigaciones Biológicas, Madrid, Spain). Collagen type I laminin, poly-l-lysine, and fibrinogen were purchased from Sigma (St. Louis, MO). BSA was obtained from Boehringer Mannheim (Mannheim, Germany).

Cells

IL-2 cultured NK cells were obtained essentially as previously described (32). In brief, PBL were cultured with irradiated (5 Gy) RPMI 8866 lymphoblastoid cells for 6 to 9 days in RPMI 1640 supplemented with 10% FCS (complete medium), followed by a negative selection step using an anti-CD3 mAb plus rabbit complement (Behring, Marburg, Germany). The CD3+ cells (<5% CD3+) were cultured with 50 IU/ml of rhIL2 until use. Fresh NK cell-enriched populations were obtained from PBL by removal of adherent cells on plastic petri dishes, followed by passage through a nylon wool column, and depletion of T cells with an anti-CD3 mAb plus rabbit complement (32). These cell populations are hereafter referred to as NK cells. After each purification process, the resulting population was characterized by flow cytometric analysis. We routinely obtained a cell population with percentages of CD56+ and CD16+ cells >95% and with <5% of CD3+, CD19+, or CD14+ contaminating cells. For polarization studies, NK cells were cultured in the absence of exogenous IL-2 for 12 h before the experiment. Erythroblastic K562 target cells were cultured in complete medium.

Flow cytometric analysis

Cells were saturated with γ-globulin, incubated with the appropriate mAb for 30 min at 4°C, then washed twice and incubated with a 1/50 dilution of FITC-labeled rabbit F(ab')2 anti-mouse IgG (Pierce, Rockford, IL) for 20 min at 4°C. Double-staining experiments on unfraccionated PBMC were performed using phycoerythrin-labeled anti-CD56 mAb after incubation with 10% mouse serum. Flow cytometric analysis was performed using a FACScan cytometer (Becton Dickinson, Mountain View, CA).

Immunofluorescence analysis

The induction and detection of the uropod on NK cells were performed essentially as previously described (28). Briefly, 1 × 10^6 NK cells were incubated on coverslips coated with various protein substrates in flat-bottom 24-well plates (Costar, Cambridge, MA) in a final volume of 500 ml of complete medium. Chemokines and other cytokines were added at different concentrations, and cells were allowed to settle for 30 min at 37°C in a 5% CO2 atmosphere. Cells were then fixed with 3.7% formaldehyde in PBS for 10 min at room temperature, rinsed in PBS (50 mM Tris-HCl (pH 7.6), 150 mM NaCl, and 0.1% NaN3), and incubated with specific mAbs. After washing, cells were stained with a 1/50 dilution of an FITC-labeled rabbit F(ab')2 anti-mouse IgG (Pierce) and analyzed using a Nikon Labophot-2 photomicroscope (Nikon, Melville, NY) with ×40, ×60, and ×100 oil immersion objectives. The proportion of uropod-bearing cells was calculated by direct counting of total cells (n = 400–500) and uropod-bearing cells in 10 random fields (×60 objective) for each condition. Preparations were photographed on Ektachrome 400 film (Eastman Kodak, Rochester, NY).

For immunofluorescence studies of NK-K562 cell conjugates, 10^5 K562 cells and 2 × 10^5 NK cells were mixed and incubated in Ca2+-free complete medium (ICN, Costa Mesa, CA), to avoid cytolysis, at 37°C in a 5% CO2 atmosphere for 20 min in 96-well plates (Costar). Cells were then fixed with 3.7% formaldehyde, rinsed in Tris-buffered saline (TBS), stained with mAb, washed twice with TBS, and sequentially rinsed and incubated for 15 min with a biotinylated anti-mouse IgG mAb (1/300 dilution), a fluorescein-labeled avidin D (1/1000), a biotinylated anti-avidin D (1/100), and a fluorescein-labeled avidin D (1/500; all from Vector, Bellingame, CA). CCR2 and CCR5 were stained with the appropriate mAb and then incubated with a 1/50 dilution of an FITC-labeled rabbit F(ab')2 anti-mouse IgG (Pierce). Cells were observed and photographed using a Nikon Labophot-2 photomicroscope with ×40 and ×60 oil immersion objectives.

Time-lapse videomicroscopy

Videomicroscopy analysis was performed as previously described (35) using a Nikon Diaphot 300 inverted microscope equipped with a black and white video camera (Sony SSC-M350CE) coupled to a time-lapse videocassette recorder (Sony SVT-5000P) and a video monitor (Sony PVM-1453 MD). NK cells were allowed to attach for 30 min at 37°C to

FIGURE 1. Chemokine production and chemoattraction of NK cells during NK-K562 cell interaction. A. Chemoattraction of NK cells during NK-K562 cell interaction. IL-2-activated NK and K562 cells, either alone or mixed, were cultured in the lower well of a transwell chamber for 4 h. Cells were incubated in Ca2+ free complete medium; migration of cells was calculated as described in Materials and Methods. The arithmetic mean ± SEM of three independent experiments with different donors is shown. *, p < 0.05 compared with cell migration in response to the medium; **, p < 0.05 compared with cell migration in response to NK cells (as determined by Student’s t test). B. Chemokine production induced by NK-K562 conjugate formation. IL-2-activated NK and K562 cells, either alone or mixed, were incubated in complete medium for 4 h. Then, cell supernatants were assayed for different chemokines as described in Materials and Methods. The arithmetic mean ± SEM of two independent experiments with cells from two different donors are shown.
10-mm plastic petri dishes (Costar) previously coated with ICAM-1 (10 mg/ml), in the presence of different chemokines or the polarization-inducing anti-ICAM-3 mAb HP2/19. A second cohort of unstimulated NK cells was then added, and cell-cell interactions were filmed for 1 h using a 3x20 phase contrast objective. When indicated, cells were pre-treated for 15 min at 37°C with different blocking mAb. Images were acquired every 30 s, and sequential frames were photographed. Under each experimental condition, the number of attached cells from the first cohort that were moving on the substrate (phase-dark cells) was counted as well as the number of cells from the second cohort (phase-bright cells) that were interacting with the uropod of adhered cells. The recruitment index was expressed as the number of cells of the second cohort being captured/number of the cells of the first layer that adhered to the substrate.

Chemotaxis transwell assays

Chemotaxis assays were performed in triplicate in transwell cell culture chambers (Costar). These chambers contain an upper and a lower well separated by a tissue culture-treated polycarbonate membrane (polypyrrolidone free), 6.5 mm in diameter, 10 mm thick, and with a pore size of 5 µm. K562 target cells (5 x 10^5), NK cells (5 x 10^5), or a mixture of both cells were preincubated in a final volume of 0.6 ml of complete medium for 4 h at 37°C in 5% CO₂. Cells were then centrifuged, and the chemokines present in the supernatant were quantified. Human chemokines MCP-1 and RANTES were measured using the Cytoscreen immunoassay kit (BioSource, Camarillo, CA), and human MIP-1α and MIP-1β were determined using the Quantikine immunoassay kit (R&D Systems, Minneapolis, MN), following the manufacturer’s instructions.

Cytotoxicity assays

NK cell cytotoxicity was tested against the K562 cell line in a 4-h ⁵¹Cr release assay at different E:T cell ratios, and specific lysis was calculated as previously described (32). Data are expressed as the arithmetic mean of triplicate determinations. In each case, spontaneous release was always <10% of the maximum lysis.

Statistical analysis

Significant values were determined using one-tailed Student’s t test.

Results

**NK-target cell cocultures release chemokines and attract additional NK cells**

Chemokines are produced by different cell types, including NK cells (15–17). We analyzed whether NK cells, alone or mixed with target cells, release soluble factors that attract additional NK cells. Using a chemotaxis transwell assay, we found that cocultures of
NK cells with K562 target cells induced a noticeable migration of additional NK cells, whereas K562 or NK cells alone had a weak effect (Fig. 1A). Measurement of chemokines in these supernatants also revealed that NK cells produced MIP-1α, RANTES, and MIP-1β, but not MCP-1, whereas only trace amounts of these chemokines were found in K562 cell supernatants. Interestingly, the secretion of these chemokines, probably by NK cells, was dramatically increased upon coculture of NK effectors with K562 target cells (Fig. 1B). These results indicate that the interaction of NK cells with target cells induces the release of physiologically relevant amounts of chemokines.

**Chemokines induce the formation of two specialized domains in NK cells**

Membrane expression of CCR2, a receptor for MCP-1, MCP-3, and MCP-4 (36), and CCR5, which specifically binds RANTES, MIP-1α, and MIP-1β (37), was barely detectable on freshly isolated NK cells (Fig. 2A). In contrast, CCR2 and CCR5 expressions were up-regulated in a significant proportion of IL-2-activated NK cells (Fig. 2B). IL-15, which has been reported to be a key cytokine in the development of NK cells (38, 39), showed a comparable effect on the induction of expression of these two chemokine receptors (Fig. 2C). These results are consistent with studies of the up-regulated CCR2 mRNA expression in NK cells (40).

We have reported that chemokines induce the formation of two specialized cell compartments in T cells; while chemokine receptors cluster at the leading edge of migrating T cells, adhesion molecules (ICAM-1, ICAM-3, CD43, and CD44) redistribute to the cell uropod (26, 28). We therefore explored the roles of chemokines in regulating NK cell morphology and receptor distribution. The chemokines RANTES, MCP-1, and, to a lesser extent, MIP-1α and MIP-1β induced polarization of both CCR2 and CCR5 to the leading edge of IL-2-activated NK cells. Neither the proinflammatory cytokine TNF-α nor the activating molecule PMA induced CCR polarization. Two integrin ligands, FN80 and ICAM-1, supported CCR redistribution, which is consistent with our previous data indicating that receptor polarization requires adhesion through integrins (Fig. 2, b and c). Chemokines also triggered the formation of the uropod and ICAM-3 redistribution on IL-2-activated NK cells in a dose-dependent manner (Fig. 3, a–c).
Although MIP-1α and MIP-1β induced the uropod in a lower proportion of cells than did RANTES and MCP-1, these chemokines showed a significant effect at lower concentrations (0.01–1 ng/ml; Fig. 3b). IL-15, known to be a chemotactic factor, also induced the redistribution of ICAM-3 to the uropod of NK cells and the chemokine receptors to the leading edge (Fig. 3c and data not shown) (26, 27, 29). Redistribution of CCR and adhesion receptors hence correlated with the acquisition of the migratory morphology and may guide cell migration and cell-cell interactions during NK cell effector function.

Role of cell polarization during NK-target cell conjugate formation

We next studied the role of the uropod and the leading edge in NK cell motility, and the formation of effector-target cell conjugates by time-lapse microscopy. IL-2-activated NK cells migrated onto FN80-coated surfaces; they show a polarized shape, with the uropod at the rear of the cell and the leading edge at the opposite pole. Motile NK cells contacted the K562 target cells through the leading edge, maintaining their polarized shape (Fig. 4a). Contacts through the uropod were rarely observed, indicating that the existence of two morphologic poles correlates with different functional domains.

To further assess the role of cell polarization during the NK cell cytotoxic response, we studied the redistribution of CCR and adhesion molecules during the formation of NK-target cell conjugates. The IL-2-cultured NK cells exhibited a polarized morphology when bound to K562 target cells (Fig. 4b). In these immunofluorescence studies, NK cells were clearly distinguished from K562 cells based on their polarized cell shape, lower refringence, and smaller size. CCR2 and CCR5 were localized at the area of target-effector cell-cell contact (Fig. 4b, A–E). In contrast, ICAM-3 and ICAM-1 were concentrated in the uropod, at the opposite side of the cell-cell contact area (Fig. 5a, A–D). Since K562 cells express ICAM-1, but not ICAM-2, ICAM-3, or VCAM-1, it is likely that NK cell polarization as well as binding to K562 target cells are mediated through the LFA-1/ICAM-1 adhesion pathway (41). Although LFA-1 is evenly distributed, it shows a reinforced location at the cell-cell contact areas (Fig. 5a, insets in E and F). This was further
confirmed using the L16 mAb, which reacts specifically with an activation-dependent epitope on the LFA-1 \(\alpha\)-chain. This Ab stained the cell-cell contact areas, but not the uropod region (Fig. 5a, E and F). In contrast, none of the NK cell-specific receptors, such as CD56, CD16 (Fc\(\gamma\)RIII), or CD94, accumulated at the uropod; but they were evenly distributed throughout the cell membrane (Fig. 5b, A–F). These results show that upon target cell conjugate formation, NK cells appear to maintain a polarized shape, implying the formation of specialized functional domains on the cell membrane.

**Chemokines induce uropod-mediated recruitment of NK cells: involvement of ICAM-3 and relevance in effector-target cell conjugate formation**

The fact that the uropod concentrates several adhesion molecules in a highly exposed region of the cell prompted us to study the role of this structure in homotypic adhesion and NK cell recruitment. We induced cell polarization and adhesion receptor redistribution in a layer of NK cells adhered to ICAM-1-coated plates. Unstimulated NK cells were

![Image](http://www.jimmunol.org/)

**FIGURE 5.** Redistribution of adhesion molecules in NK-target cell conjugates. *a*, Redistribution of ICAM-1 and -3 to the uropod. ICAM-3 (A and B) and ICAM-1 (C and D) are concentrated on the uropod of polarized NK cells bound to K562 targets, while LFA-1 is found uniformly throughout the cell (E and F). Insets in E and F show the staining of the activation-dependent epitope of the \(\alpha\)-chain of LFA-1. *b*, Cellular distribution of the NK cell-associated receptors. Cellular distributions of CD94 (A and B), CD56 (C and D), and CD16 (E and F) on NK-target conjugates are shown. Bar = 10 \(\mu\)m.

**FIGURE 6.** Uropod-bearing NK cells recruit other NK cells. NK cells were allowed to bind to plastic petri dishes coated with ICAM-1-Fc and then were incubated with RANTES (10 ng/ml) for 30 min at 37°C. After addition of a second cohort of untreated NK cells, cells were filmed with a time-lapse videocassette recorder for 1.5 h. Time frames obtained from one representative experiment are shown. Arrowheads indicate cells from the second cohort (bright cells) bound to the uropod of migrating NK cells (dark cells). Small arrows indicate the direction of the leading edge of the cell. A schematic representation of the cell movement is also shown.
then added, and cell-cell interactions were video recorded. Following treatment with RANTES (Fig. 6), the NK cells from the first layer migrate onto the substrate (phase-dark cells), contact unstimulated NK cells (phase-bright cells) through the uropod, and capture and transport them, thus moving together. These captured cells were tightly attached through the uropod, and the cell transport phenotype was long lasting, persisting for at least several minutes. Quantitative analysis of the chemokine-induced NK cell recruitment showed that MCP-1 and RANTES clearly enhanced this phenomenon, whereas MIP-1α and MIP-1β had weaker activity, and TNF-α had no significant effect (Fig. 7A). The strongest response was induced by the polarization-inducing anti-ICAM-3 mAb HP2/19 (Fig. 7). The effects of MCP-1 and the anti-ICAM-3 HP2/19 mAb on cell recruitment were specifically inhibited with the anti-ICAM-3 blocking mAb D3/9 (Fig. 7, B and C). These results show the involvement of chemokines in NK cell recruitment and the dependence of this phenomenon on ICAM-3.

We next studied the role of NK cell recruitment on effector-target cell conjugate formation. NK cells adhered to FN80 were induced to polarize by MCP-1 (Fig. 8). A second cohort of non-stimulated NK cells was then added, and after 30 min, K562 cells were added, and NK cell migration was filmed. As described above, uropod-bearing NK cells tightly trapped NK cells from the second cohort, which were guided by the polarized migrating NK cells to contact the K562 cell (Fig. 8). This finding suggests that during the cytotoxic response, adhesion receptor redistribution to the uropod may exert an amplifying effect on cell conjugate formation by increasing the number of NK cells available to interact with target cells.

**Prevention of cell polarization and receptor redistribution inhibits NK cell-mediated cytotoxicity**

We have reported that disruption of the myosin motor by specific drug inhibitors prevents cell polarization and redistribution of adhesion receptors to the cell uropod without affecting cell adhesion (42, 43). To further analyze the role of cell polarization and adhesion receptor redistribution in the cytotoxic activity of NK cells, we investigated the effects of the myosin-disrupting agent butanedione monoxime in effector-target conjugate formation as well as in NK cell-mediated lysis of K562 target cells. Butanedione monoxime, which inhibited NK cell polarization and ICAM-3 redistribution to the cell uropod (Fig. 9aB), prevented conjugate formation (Fig. 9aF and G) and blocked NK cell-mediated cytotoxicity (Fig. 9b). In contrast, nocodazole, which disassembles the microtubule network but did not inhibit uropod formation and cell polarization (Fig. 9aC (44), did not alter the formation of cell conjugates (Fig. 9aH and I). NK cell cytotoxicity mediated by cells with disassembled microtubules was only weakly influenced, in accordance with a previous report (Fig. 9b) (45). These results suggest that chemokines may affect NK cell-mediated target cell conjugate formation and cytotoxicity by regulating receptor redistribution and cell polarization.

**Discussion**

NK cell motility and NK-target cell conjugate formation are limiting steps in NK cell cytotoxic activity. Chemokines and some chemoattractant cytokines such as IL-15 have been described as modulators of NK cell motility and cytotoxic activity (11–14, 46, 47). When migrating, lymphocytes adopt a polarized phenotype, displaying a cytoplasmic projection at the rear of the cell, termed uropod due to its similarity with ameboid morphology (20, 48). Here we show that chemokines and IL-15 also induce the polarization of NK cells bound to endothelial or extracellular matrix proteins, a phenomenon that is accompanied by redistribution of adhesion receptors (ICAM-1 and ICAM-3) to the uropod. We have also found that the chemokine receptors CCR2 and CCR5 are redistributed to the leading edge of NK cells, and that this chemokine receptor distribution correlates with the acquisition of a migratory phenotype. Our results using time-lapse video studies confirm that...
FIGURE 8. NK cells recruited by uropod-bearing NK cells are driven to contact target cells. NK cells pretreated with the chemokine MCP-1 capture other NK cells through the cellular uropod and subsequently transport them to the vicinity of target cells. NK cells were allowed to bind to plastic petri dishes coated with FN80 and then were incubated with MCP-1 (10 ng/ml) for 30 min at 37°C. After addition of a second cohort of untreated NK cells and the K562 target cells, cells were filmed with a time-lapse videocassette recorder for 1.5 h. Time frames obtained from one representative experiment are shown. Arrowheads indicate NK cells from the second cohort bound to the uropod of the migrating NK cells. Small arrows indicate the direction of the leading edge of the cell. T and E indicate target and effector cells, respectively. A schematic representation of the cell movement is also shown.

uropod-bearing NK cells correspond to migrating cells, further indicating the association between uropod formation and leukocyte migration (20, 28, 35, 48). It therefore seems feasible that the CCR cluster at the advancing front may function as a mechanism to guide the NK cell to mediate cell-cell interactions.

Cell polarization plays an important role in the immune response as well as in other biologic processes (21–24, 27). For instance, T cells recognize and bind APCs through their leading edge (21, 23). Using time-lapse videomicroscopy, we observed a similar behavior for NK cells when they interact with the K562 target cells; NK cells contacted the target cells through the advancing front, a region in which CCR2 and CCR5 are concentrated, whereas the effector-target cell interactions through the uropod were rarely observed. We also found that during the formation of effector-target cell conjugates, ICAM-1 and ICAM-3 adhesion molecules are concentrated in the uropod of NK cells, which is located at the most distal point from the area of NK-target cell contact. The redistribution of adhesion molecules and CCR to the opposite poles of NK further supports the idea that cell polarization arises in two specialized regions on the cell membrane, which make feasible several key phenomena, such as cell migration and intercellular adhesion. The functional relevance of cell polarization is made evident by the fact that both CCR and adhesion receptor redistribution are maintained not only during NK cell migration, but also when they are bound to their targets. Furthermore, preventing cell polarization and adhesion receptor redistribution blocked the formation of effector-target cell conjugates and NK cell-mediated cytotoxicity, thus demonstrating the functional importance of these phenomena.

The mechanisms by which NK cells promote cytotoxic activity in specific tissues remains poorly understood. We propose the existence of a cooperative mechanism involved in NK cell migration. On the one hand, by inducing cell polarization, chemokines and cytokines play important roles in the development of the NK cell cytotoxic response. Cell polarization determines the formation of different functional domains in the NK cell. The leading edge, which concentrates the CCR, is involved in target cell adhesion, granule release during cytotoxic phenomena (25), and probably directing the cell during migration, whereas the uropod, which accumulates ICAM, is involved in leukocyte recruitment. In fact, uropod-mediated cell-cell contacts appear to recruit additional NK cells to the vicinity of the target cells, which may increase contact frequency between NK and target cells, favoring activation of the recruited cells. This concurs with our earlier studies of T lymphocytes, in which ICAM redistribution to the uropod is a cooperative mechanism in lymphocyte recruitment, acting as an amplification system during both cell extravasation and migration toward inflammatory foci (28, 35). On the other hand, the molecules inducing NK cell polarity appear to be chemokines produced by activated NK cells. A role for chemokines in the activation of NK cell-mediated cytotoxicity has also been previously reported (12, 13). In agreement with our findings, the authors found that the enhancement of NK cytotoxicity mediated by chemokines was dependent on conjugate formation (12). We have found that the formation of NK-target cell conjugates induces the release of chemokines, which are responsible for NK cell migration, a finding that further substantiates our hypothesis of a cooperative mechanism of NK cell migration to the target. Some of these events, including cell polarization and the induction of cytotoxic activity, can be blocked by ADP ribosylation of the GTP binding protein RhoA, indicating the key role of this chemokine receptor-associated signaling pathway (49). In summary, these observations are consistent with the view of the NK cell as a sensitive immune cell, responsive to external cues and able to alter its shape in response to diverse stimuli.

As occurs in vitro, it is also conceivable that the in vivo interaction of NK with target cells may induce the release of soluble factors, including chemokines probably produced by effector cells, which can trigger NK cell polarization, chemotaxis, and recruitment of additional NK cells to the tissue. It is thus entirely feasible that the in vivo recruitment of NK cells triggered by chemokines and cytokines at sites of immune activation has an important amplifying effect in the elimination of tumor cells and parasites. The induction of NK cytotoxicity by IL-15 secreted during human herpes virus infection may be an example of such a mechanism (47). IL-15 acts as a chemotactic factor that induces cell polarization (27) and adhesion receptor distribution (29) and regulates the migration of lymphocytes at inflammation sites in diseases such as rheumatoid arthritis (50). Our data suggest that chemokines have a similar in vivo role in NK cell-mediated phenomena.
FIGURE 9. Inhibition of conjugate formation and NK cell cytotoxicity by a myosin-disrupting drug. a, Conjugate formation is inhibited by disruption of myosin-motor. A to C correspond to immunofluorescence studies showing ICAM-3 distribution and cell shape morphology of RANTES-stimulated NK cells, untreated (A) or pretreated for 30 min with either 20 mM butanedione monoxime (B) or 10 μM nocodazole (C). The effects of these cytoskeleton-disrupting agents on NK-target cell conjugate formation are shown in D to I. IL-2-cultured NK cells were incubated for 30 min in the absence (D and E) or the presence of 20 mM butanedione monoxime (F and G) or 10 mM nocodazole (H and I). Then, cells were allowed to adhere to FN for 30 min at 37°C. K562 target cells were added, and the cytotoxic process was video recorded for 1 h. Two sequential representative video frames of each experimental condition are shown. To illustrate, some targets (T), polarized NK cells bound to the targets (arrowheads), or effector cells (E) are indicated. Note the round shape of NK cells treated with butanedione monoxime (F and G). b, Effect of the myosin disruption drug butanedione monoxime on NK cell cytotoxicity. NK cells were incubated in the absence or the presence of 20 mM butanedione monoxime, 10 μM nocodazole, or control solvent for 30 min at 37°C. Then, NK cell killing activity was determined in a 4-h 51Cr release assay, as described in Materials and Methods. The arithmetic mean ± SD of triplicate determinations is shown. A representative experiment of three performed with cells from different donors is shown.

Acknowledgments
We thank Dr. J. P. Albar for help in peptide design for the production of anti-CCR5, C. Mark for editorial assistance, C. Cabanas and M. Montoya for assistance with the time-lapse videomicroscopy studies, and R. Tejedor and M. Vitió for technical support.

References
interacts with LFA-1 and regulates the LFA-1/ICAM-1 cell adhesion pathway.


