Ramoni, C. et al. Differential expression and distribution of ezrin, radixin and moesin in human natural killer cells. Eur. J. Immunol. 32, 3059–3065

ARTICLE *in* EUROPEAN JOURNAL OF IMMUNOLOGY · NOVEMBER 2002

Impact Factor: 4.03 · DOI: 10.1002/1521-4141(200211)32:11<3059::AID-IMMU3059>3.0.CO;2-3 · Source: PubMed

Differential expression and distribution of ezrin, radixin and moesin in human natural killer cells

Carlo Ramoni, Francesca Luciani, Francesca Spadaro, Luana Lugini, Francesco Lozupone and Stefano Fais

Laboratory of Immunology, Istituto Superiore di Sanità, Rome, Italy

Cytoskeleton plays a crucial role in natural killer cell function. In this study the expression and subcellular distribution of ezrin, radixin and moesin, a family of proteins that connect actin filaments to many membrane structures, were evaluated in human NK cells. The results showed that NK cells expressed all these proteins, while NK cell-deprived peripheral blood leukocytes and purified T lymphocytes did not express radixin. Only ezrin changed its distribution following IL-2 activation and all three ezrin, moesin and radixin were polarized on uropods of adherent natural killer cells. Ezrin and radixin co-localized with the perforin granules at the intimate sites of contact between NK and the target cells, while moesin remains uniformly distributed on the membrane of NK cells. Ezrin, radixin and perforin co-localization was undetected in non-lytic conjugates and inhibited by treatment with actin depolymerizing agents. These results suggest that ezrin and radixin may exert a role in NK activity, particularly in the trafficking of perforin granules to the NK/target cells contact site. Moreover, our data suggest that radixin may represent an additional biological marker of human NK cells and that this protein may hold a specific role in NK cell function.

Received	14/3/02
Revised	11/7/02
Accepted	29/8/02

Key words: NK / Ezrin / Radixin / Moesin / Perforin

1 Introduction

Evidence is accumulating that cytoskeleton plays a crucial role in NK cell function. Particularly, actin filaments seem to drive both polarization of single NK cells and the formation of the immunological synapse between NK cells and the target cells [1–4]. NK cell polarity was intrinsically involved in conjugate formation [5–6]. This cytoskeletal reorganization precedes and could be a prerequisite for the polarization of the cellular secretory apparatus and the polarized release of cytotoxic vesicles, essential for cytolytic activity against their target cells, and may be functionally responsible for the required cytokinetic movements [6–8]. However, the direct interaction between actin and NK granules has been poorly explored. Ezrin, radixin and moesin (ERM) belong to a family of proteins that connect some membrane proteins to the actin cytoskeleton [9–11]. The linkage of membrane proteins with the actin cytoskeleton allows ERM to participate to the formation of membrane structures like microvilli, lamellipodia, filopodia and uropodia [9]. Of interest, the specific inhibition of ERM synthesis and function both results in loss of membrane structures and profoundly affects some crucial cellular activities [9–12]. Expression, distribution and function of ERM in NK cells have been poorly explored. Thus, in this study the expression of ezrin, radixin and moesin in human NK cells was evaluated by reverse transcriptase RT-PCR, Western blot and confocal microscopy. Moreover, the subcellular distribution of ERM was investigated in resting, lymphokine-activated and adherent NK cells, as well as in NK/target cell conjugates. Finally, the cellular localization of ERM was compared to that of perforin granules in the NK cell/target cell contact site. The results showed that natural killer cells expressed all the ERM, including radixin, while NK cell-deprived peripheral blood leukocytes and purified T lymphocytes did not express radixin. The distribution of ERM was highly polarized on uropods of adherent NK cells. However, ezrin fully co-localized with the perforin granules at the intimate sites of contact between natural killer and the target cells. Radixin showed a thin area of co-localization in the intimate contact sites, while moesin remained uniformly distributed on the membrane of natural killer cells. Moreover, ezrin, radixin and perforin co-localization was not detected in non-lytic conjugates and treatment with the actin depolymerizing agent latrunculin-A markedly inhib-

[I 23022]

The first two authors equally contributed to this work.

Abbreviations: ERM: Ezrin, radixin and moesin **RT-PCR:** Reverse transcriptase PCR **CLSM:** Confocal laser scanning microscopy **ALAK:** Adherent LAK

 0014-2980/02/1111-3059$17.50+.50/0

ited the co-localization of these molecules in the NK/target cells contact sites.

2 Results

2.1 ERM expression in human NK cells

ERM expression was evaluated in purified NK cells by both RT-PCR and Western blot analyses. The results showed that NK cells expressed transcripts for all the ERM, while NK-deprived PBL and purified T lymphocytes exclusively expressed ezrin and moesin transcripts, independently from the state of activation of the various cell subpopulations (Fig. 1A). Densitometric analysis of Western blot films (normalized on β-tubulin expression levels) (Fig. 1A) revealed that the ERM expression is comparable in each cell subpopulation, independently from the state of activation (not shown). Western blot analysis showed results comparable to the RT-PCR observations (Fig. 1B). This set of results suggested that radixin was a specific marker of NK cells, and that a specific role of this protein in NK cell function was highly conceivable.

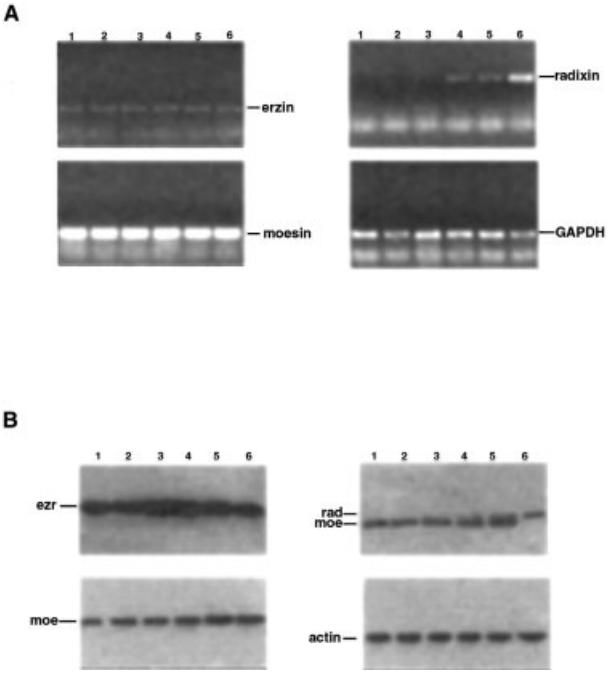

Fig. 1. ERM expression in NK cells. (A) RT-PCR analysis of ezrin (ezr), radixin (rad) and moesin (moe) expression in NK cells. Resting (1) and activated (2) purified T cells and NK-depleted PBL (3) were compared to resting (4) and IL-2 activated (5) NK cells. In (6) positive controls are shown. (B) Western blot analysis of the same samples. GAPDH and actin (act) levels in the relative lysates are shown.

2.2 ERM distribution in resting, activated and adherent NK cells

Thus, we investigated the relative distribution of ERM in either resting, IL-2-activated killer cells (LAK) and adherent-LAK (A-LAK) cells by confocal microscopy. The results showed that NK cell IL-2 activation induced straightforward changes in the distribution of ezrin that accumulated in microvillar structures of activated NK cells (Fig. 2, upper panels), while radixin and moesin did not appear to modify their distribution following IL-2 stimulation (Fig. 2, central and lower panels, respectively). To further explore the changes in ERM distribution we obtained A-LAK cells, following potent IL-2 stimulation, analyzing the staining for the ERM in these cells. The results showed that A-LAK cells displayed marked changes in the distribution of the ERM that appeared all extremely polarized on uropods (Fig. 2, right panels). Particularly, ezrin and radixin appeared unidirectionally polarized in giant uropods, while moesin even mainly localized on uropods, was ubiquitously localized on the juxtamembrane region of these cells.

2.3 ERM distribution in NK/target cells conjugates

To further investigate a possible involvement of ERM in the NK function we cultivated NK cells in the presence of classical target cells (K562 cells), evaluating the distribution of ERM in the NK/target cells conjugates, as compared to the distribution of perforins. The results first showed the absence of ERM/perforin granules co-localization in single NK cells (Fig. 3A, E, I). However, the experiments performed with the NK/target cells conjugates demonstrated that (i) ezrin co-localized with perforins in the NK cell-to-target cell contact site (Fig. 3B-D); (ii) radixin was markedly polarized in the cell-to-cell contact site, while partially co-localizing with perforins in this area (Fig. 3F-H); and (iii) moesin remained uniformly distributed on the NK cell membrane and did not show polarization or co-localization with perforins in the cell-to-cell contact sites (Fig. 3L-N). Ezrin and radixin co-localization with perforins was further investigated in serial optical sections of the NK/target cells contact sites. The results showed that ezrin and perforins are markedly polarized in NK cells, where they entirely co-localize in the NK cell/target cell contact sites (Fig. 4A). Radixin was more widely distributed in NK cells, although co-localizing with perforins in the site of the intimate contact between the two cells (Fig. 4B).

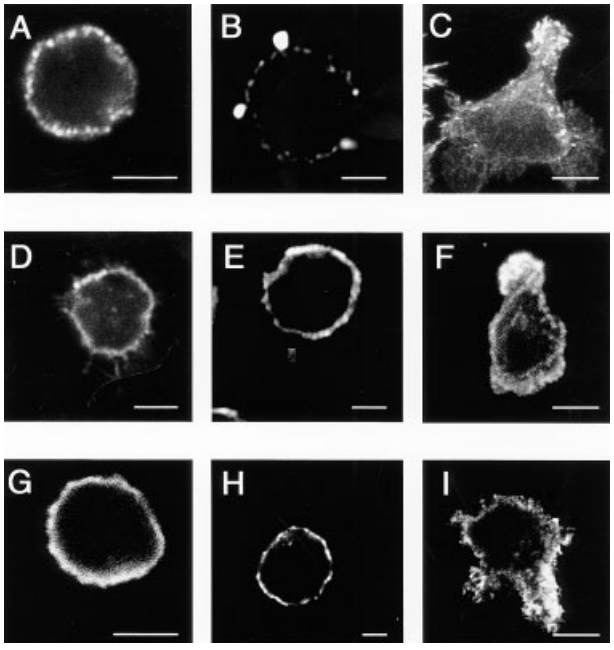

Fig. 2. CLSM analyses of the relative expression and localization of ERM proteins. Resting (left panels), IL-2-activated (central panels) and adherent NK cells (right panels) from the same donor are shown. Expression and localization of ezrin (A, B, C), radixin (D, E, F) and moesin (G, H, I) are detected by specific Ab. A packed of three central confocal sections (0.7 μm each) are shown. These results were confirmed by four independent series of experiments (scale bar: 5 μm).

We thus performed experiments aimed at evaluating the role of both ezrin and radixin in the trafficking of perforin granules and the importance of ERM/perforin co-localization in NK function. To investigate the role of ezrin and radixin in the traffic of perforin granules we treated NK cells with latrunculin-A, an actin cytoskeleton disrupting agent, evaluating by confocal microscopy the reciprocal distribution of ERM and perforin granules in NK/target cells conjugates. Latrunculin-A pre-treatment dramatically inhibited both the polarization of ezrin, radixin and perforin granules and their reciprocal co-localization at the NK/target cells contact sites (Fig. 4C), thus confirming a key role for actin cytoskeleton in cytolytic granules trafficking and unidirectional polarization, and therefore, supporting a pivotal role for ezrin and radixin in the polarized transport of cytolytic granules. These observations were strengthened by the analysis of ezrin, radixin and perforin distribution in non-cytolytic conjugates formed by NK and DAUDI cells. The results showed that ezrin, radixin and perforin granules did not polarize nor co-localize at the NK/DAUDI cells contact sites (Fig. 4D).

3 Discussion

Many aspects of the direct interaction of NK cells with the target cells leading to the cytotoxic effect were still unknown. The actin cytoskeleton exerts a key role in the early phases of the NK cell/target cell conjugation [2–4, 6] and a straightforward polarization of the actin filaments occurs in NK cells before and during contact with the target cells [4]. Moreover, direct or indirect inhibition of cytoskeleton impairs NK cell activity [2, 4, 13]. In this study we have investigated the implication of proteins belonging to the ERM family in NK cell activity. The results of our study show that ERM are markedly expressed in NK cells. Notably, radixin is not expressed by lymphocytes deprived by NK cells and in purified T cells, independently from their state of activation, supporting previous data showing that radixin was mostly undetectable in human lymphocyte preparations [10, 14]. Our data show for the first time that NK cells are the only circulating lymphocyte subpopulation that express radixin, in turn suggesting that radixin may exert a specific role in NK cell function. In further exploring this point we have investigated the specific localization of the ERM in various *in vitro* activation/differentiation conditions of NK cells. The results showed that only ezrin changed its distribution, localizing in microvillar structures of NK cells, while radixin and moesin remained uniformly distributed on the cell membrane. This finding seemed not surprising, as following activation, ezrin localizes to microvillar structures in many cell types [15, 16]. Potent IL-2 stimulation induces NK cells to adhere to various substrates including glass, plastic and extracellular matrix components (A-LAK) [17]. A-LAK cells were described as motile, and polar, with a ruffled leading edge at one end of the cell and a uropod at the opposite end, with a high degree of internal polarity, with the nucleus at the leading edge of the cell and richly granulated cytoplasm at the uropod [17]. Extensive cytoskeletal structures, characterize these cells expressing numerous podosomes and larger adherence structures containing polymerized actin, which appear to be important for interactions of these cells with the substrate. Our data are consistent with this early description of A-LAK cells, suggesting a role of these three closely related proteins, in migration, adhesion and cell-to-cell communication of NK cells within tissues. When we analyzed the ERM distribution in NK/target cell conjugates only ezrin and radixin proved to be concentrated in the intimate cell-to-cell contact sites. This suggested a specific role of ezrin and radixin in NK cell function. In fact, radixin and ezrin have redundant function and are coexpressed in many cell types as a safety measure [18]. However, the effects of the loss of radixin may be more pronounced in neurons compared with other cells [19], suggesting that ERM may exert tissue-specific functions. Differences in

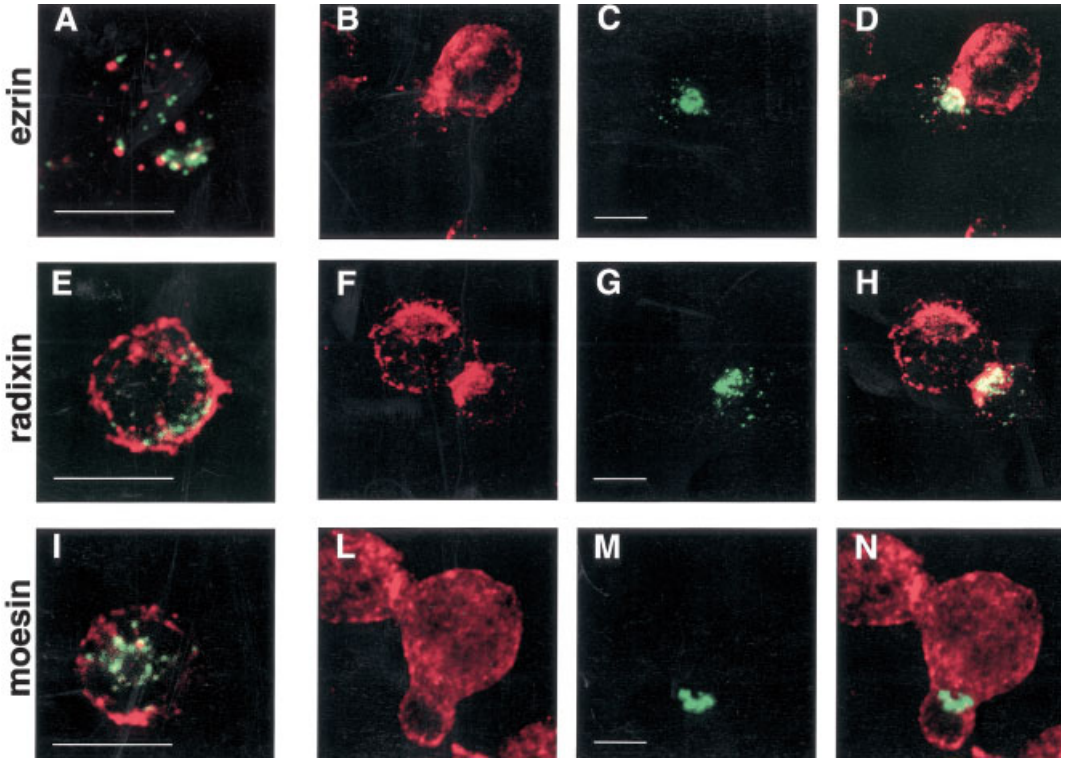

Fig. 3. Co-localization of ERM proteins with perforin molecules as marker for NK-K562 cell conjugates. Dual fluorescence CLSM analyses were performed first on fixed single-activated NK cells, double-stained with anti-ezrin (A), -radixin (E), or -moesin (I) polyclonal Ab and anti-human perforin mAb. Dual fluorescence CLSM analyses were thus performed on fixed activated NK/K562 conjugates double-stained either with polyclonal anti-ezrin (B, D), anti-radixin (F, H), anti-moesin (L, N), and anti-human perforin mAb (C, D, G, H, M, N). In right panels (D, H, N) ERM/perforin merges on activated NK/K562 conjugates are shown. Three-dimensional (3D) images are shown represented as both single composites and 3D reconstruction. Confocal sections, 0.7 μm apart, were taken and assembled using Multicolor Analyses software. These results were confirmed by five independent series of experiments (scale bar: 10 μm).

the use of ERM family members among different cell types may reflect distinct and restricted patterns of expression of these proteins in cell tissues and/or variations in their subcellular distribution [20, 21]. Another important issue concerning the possible involvement of actin cytoskeleton in the NK function is the cellular trafficking of the lytic granules. The secretory function of the NK cells has been considered to be essential for cytolytic activity against their target cells [22]. Notably, different types of lytic granules undergo polarized secretion and are polarized to the site of membrane contact between NK and target cells [23]. In fact, it has been shown that newly synthesized FasL is stored in specialized secretory lysosomes in NK cells and that polarized degranulation controls the delivery of FasL to the cell surface [24]. We have recently shown that perforin-containing granules clustered at the intercellular contact region of effector/target cell conjugates [25]. In this study we show that ezrin and radixin (while to a lesser extent) polarize and co-localize with perforin-containing granules, at the NK/target cell contact site. These results suggest a pivotal role of ezrin and radixin in the unidirectional polarized transport of lytic granules in NK cells. This is indirectly supported by previous data, showing that the cytoskeletal reorganization of NK cells precedes and could be a prerequisite for the polarization of the cellular secretory apparatus [6, 26]. Moreover, a role for ERM in the actin-dependent trafficking of lysosomal-like organelles has been suggested [27], and lytic granules of NK cells belong to the lysosomal-related organelles family [28]. The results of our study, showing that a potent inhibition of actin polymerization through latrunculin-A treatment induced dramatic changes in ezrin, radixin and perforin granules in the NK cells forming conjugates with the target cells further support these findings. The absence of ezrin or radixin co-localization with perforin granules in non-lytic conjugates further supported the importance of these proteins in the lytic granule-mediated NK function. Altogether, our results define for the first time the involvement of ezrin and radixin in NK cell activity and propose radixin as a possible new cytoplasmic marker of NK cells.

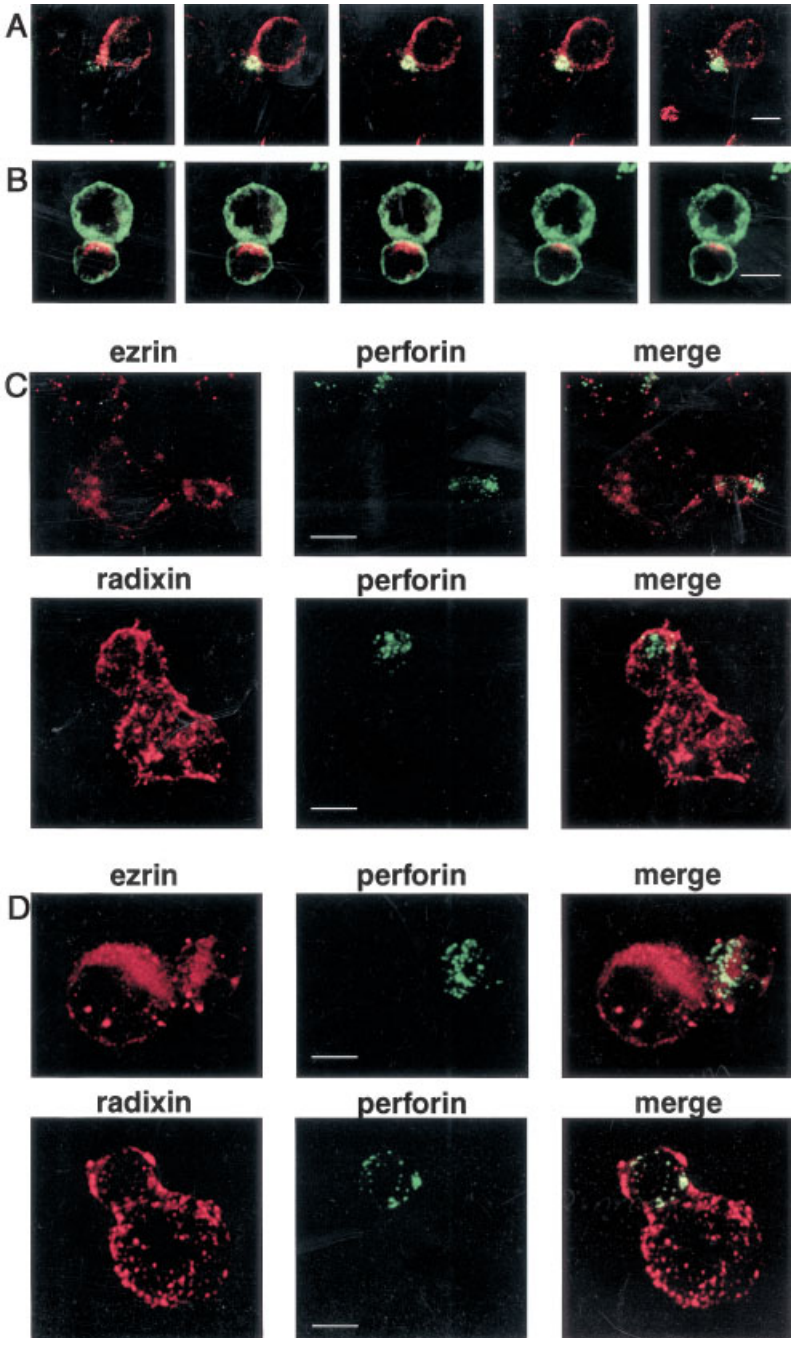

Fig. 4. Confocal analysis of ezrin and radixin co-localization with perforin granules. (A) A sequence of five optical sections, obtained by the confocal analysis of activated NK/K562 cell conjugates stained for perforin (green) and ezrin (red). The most external (left panels) to the most internal (right panels) sections of the NK-K562 contact sites are shown. The central panels show a whole overlapping of ezrin and perforin staining (yellow). (B) A sequence of five optical sections, obtained by the confocal analysis of activated-NK/K562 cell conjugates stained for radixin (green) and perforin (red). External (left panels) to internal (right panels) sections of the NK/K562 contact sites are shown. Differently to the ezrin/perforin staining only a thin area of co-localization (yellow) is detectable in the subcortical region of NK cells in central and left panels (scale bar: 10 μm). (C) Dual fluorescence CLSM analyses on activated NK/K562 cell conjugates, following pre-treatment with latrunculin-A (0.1 μg/ml, 3 h, 37°C) double-stained either with anti-ezrin (upper left panels) or anti-radixin (lower left panels) polyclonal Ab or anti-human perforin mAb (central panels). In the right panels merges are shown. (D) Dual fluorescence CLSM analyses performed on fresh NK-DAUDI conjugates, double-stained either with polyclonal anti-ezrin (upper left panels) or anti-radixin (lower left panels) or monoclonal anti-human perforin (central panels). In the right panels merges are shown. These results were confirmed by three independent series of experiments.

4 Materials and methods

4.1 Antibodies and cytokines

B73.1 (anti-CD16) was kindly provided by Dr. G. Trinchieri (Schering Plough, France). 3G8 (anti-CD16), OKT3 (anti-CD3), OKT4 (anti-CD4), OKT8 (anti-CD8) and G3D3 (anti-CD14) were produced from hybridoma cell lines (ATCC, USA) [18]. Anti-CD20, anti-CD16-PE and anti-CD56-PE were from Becton Dickinson (USA); anti-ezrin, anti-moesin and anti-radixin were from Santa Cruz (USA); and anti-human perforin mAb from PharMingen (USA). Fluorochrome-conjugated secondary antibodies: Alexa Fluor 488 and 594 goat anti-rabbit IgG $F(ab')_2$ fragment and Alexa Fluor 488 and 594 donkey anti-goat IgG were purchased from Molecular Probes, Inc. (USA). Recombinant IL-2 was from Chiron (The Netherlands).

4.2 Fresh NK cell preparation

Human peripheral blood lymphocytes (PBL) were freshly isolated from healthy donors by layering on a Histopaque-1077 (Sigma) density gradient, centrifuged and passed through nylon wool columns (Robbins Scientific Corp., USA). The NK cells were further purified by negative immunomagnetic selection. Briefly, anti-CD3, anti-CD4, anti-CD8, anti-CD20 and anti-CD14 pre-treated cells were further incubated by using magnetic particles coated with $F(ab')_2$ anti-mouse IgG (Dynal, Norway). The resulting resting NK cell population was over 80–90% $CD16^+$, $CD56^+$ as assessed by cytofluorimetric analysis.

4.3 Activated, adherent NK cells and NK/target cell conjugates

Activated NK cells were obtained by co-culturing PBL (4×10^5 cells/ml) with γ-irradiated (4,500 rad) RPMI-8866 (1×10^5 cells/ml) for 10 days, using a modified method [29]. Briefly, on day 7 of culture the medium was supplemented with recombinant human IL-2 (100 U/ml). On day 10 the contaminating T cells were removed by mixed anti-CD3, anti-CD4, anti-CD8 immunomagnetic selection. After repeated washings, the cells were further incubated with magnetic beads coated with $F(ab')_2$ anti-mouse IgG. The resulting cell population was more than 95% $CD16^+$, $CD56^+$ pure, as assessed by cytofluorimetric analysis with a panel of mAb recognizing the various lymphocyte subpopulations. A-LAK were obtained by culturing activated NK cells in medium supplemented with rIL-2 (5,000 U/ml) for further 5 days. NK/target cell conjugates were obtained by mixing (2×10^6 cells/ml) and centrifuging (5 min at 200×g) equal volumes of NK and K562 or DAUDI cells. After a 15 min of incubation at 37°C, the cell suspension was gently resuspended and incubated at 4°C for 30 min.

4.4 Isolation and activation of peripheral blood lymphocytes

Human peripheral blood mononuclear cells (PBMC) were freshly isolated from healthy donors by layering on a Ficoll-Paque density gradient. Purified $CD3^+$ T cells were isolated from PBMC by immunomagnetic negative selection using immunomagnetic beads coated with anti-CD14 and anti-CD-20 mAb (Dynal, Norway). The resultant (unbound) T cell population contained >95% $CD3^+$ cells, as assessed by flow cytometric analysis. T cell activation was performed as described [13]. At 4 and 6 days after activation cells were collected and examined for ERM expression.

4.5 Confocal laser scanning microscopy (CLSM) analyses

For CLSM analyses, NK cells and NK/target cell conjugates were seeded on poly-L-lysine-coated cover glass (10 μg/ml, 40 min, at room temperature) and allowed to attach for 15 min. Then the cells were fixed (paraformaldehyde 3%, 30 min, +4°C), washed in quenching buffer (NH_4Cl 50 mM, 10 min at room temperature) and permeabilized (TritonX-100 0.5%), 10 min at room temperature). For dual fluorescence CLSM analysis, cells were first stained with anti-ERM polyclonal Ab (30 min, +37°C) followed by donkey anti-goat-Alexa594 (30 min, +37°C) and secondly stained with the anti-human perforin mAb (30 min, +37°C), followed by goat anti-mouse-Alexa488 (30 min, +37°C). CLSM analysis was performed using a Leica TCS 4D apparatus equipped with an argon-krypton laser, double-dichroic splitters (488/568 nm), 520-nm barrier filter for AlexaFluor 488 (green), and 590-nm barrier filter for AlexaFluor 594 (red) observations. Images were acquired and processed by SCANware and Multicolor Analysis (Leica Lasertechnik GmbH, Germany) and Adobe Photoshop software programs.

4.6 RT-PCR analysis

RT-PCR for ezrin, radixin and moesin expression analysis was performed on CEM, HepG2, NK, LAK cells and NK-depleted lymphocytes as described [13]. CEM cells were used as positive control for ezrin and moesin expression; HepG2 cells were used as positive control for radixin expression.

4.7 Western blot analysis

Total cell extracts from CEM, HepG2, NK, LAK and NK-depleted lymphocyte cells were obtained as described [13]. Ezrin and moesin were detected with mAb from Transduction Labs (USA) and HRP-anti-mouse Ig (Amersham, USA). Radixin was detected with an anti-radixin goat polyclonal Ab (Santa Cruz, USA) followed by HRP-anti-goat Ig (Jackson Immunoresearch, USA) and ECL detection (Pierce, USA).

CEM cells were used as positive control for ezrin and moesin expression; HepG2 cells were used as positive control for radixin expression.

Acknowledgements: This work was supported by a grant from the Italian Ministry of Health (40/D.5 National Research Program on AIDS). F. Luciani was supported by a fellowship from F.I.R.C.

References

1 **Fassett, M. S., Davis, D. M., Valter, M. M., Cohen, G. B. and Strominger, J. L.,** Signaling at the inhibitory natural killer cell immune synapse regulates lipid raft polarization but not class I MHC clustering. *Proc. Natl. Acad. Sci. USA* 2001. **98:** 14547–14552.

2 **Poggi, A., Panzeri, M. C., Moretta, L. and Zocchi, M. R.,** CD31-triggered rearrangement of the actin cytoskeleton in human natural killer cells. *Eur. J. Immunol*. 1996. **26:** 817–824.

3 **Sconocchia, G., Titus, J. A., Segal, D. M.,** Signaling pathways regulating CD44-dependent cytolysis in natural killer cells. *Blood* 1997. **90:** 716–725

4 **Radosevic, K., van Leeuwen, M. T., Segers-Nolten, I. M., Figdor, C. G., de Grooth, B. G. and Greve, J.,** Changes in actin organization during the cytotoxic process. *Cytometry* 1994. **15:** 320–326.

5 **Nieto, M., Navarro, F., Perez-Villar, J. J., del Pozo, M. A., Gonzalez-Amaro, R., Mellado, M., Frade, J. M., Martinez, A. C., Lopez-Botet, M. and Sanchez-Madrid, F.,** Roles of chemokines and receptor polarization in NK-target cell interactions. *J. Immunol*. 1998. **161:** 3330–3339.

6 **Carpen, O., Virtanen, I., Lehto, V. P. and Saksela, E.,** Polarization of NK cell cytoskeleton upon conjugation with sensitive target cells. *J. Immunol.* 1983. **131:** 2695–2698.

7 **Kupfer, A., Dennert, G. and Singer, S. J.,** Polarization of the Golgi apparatus and the microtubule-organizing center within cloned natural killer cells bound to their targets. *Proc. Natl. Acad. Sci. USA* 1983. **80:** 7224–7228.

8 **Billadeau, D. D., Brumbaugh, K. M., Dick, C. J., Schoon, R. A., Bustelo, X. R. and Leibson, P. J.,** The Vav-Rac1 pathway in cytotoxic lymphocytes regulates the generation of cell-mediated killing. *J. Exp. Med.* 1998. **188:** 549–559

9 **Bretscher, A.,** Regulation of cortical structure by the ezrin-radixin-moesin protein family. *Curr. Opin. Cell Biol.* 1999. **11:** 109–116.

10 **Parlato, S., Giammarioli, A. M., Logozzi, M., Lozupone, F., Matarrese, P., Luciani, F., Falchi, M., Malorni, W. and Fais, S.,** CD95 (APO-1/Fas) linkage to the actin cytoskeleton through ezrin in human T-lymphocytes: a novel regulatory mechanism of the CD95 apoptotic pathway. *EMBO J.* 2000. **19:** 5123–5134.

11 **Luciani, F., Molinari, A., Lozupone, F., Calcabrini, A., Lugini, L., Stringaro, A., Puddu, P., Arancia, G., Cianfriglia, M. and Fais, S.,** P-glycoprotein-actin association through ERM family proteins: a role in P-glycoprotein function in human cells of lymphoid origin. *Blood* 2002. **99:** 641–648.

12 **Fais, S., Luciani, F., Logozzi, M., Parlato, S. and Lozupone, F.,** Linkage between cell-membrane proteins and actin-based cytoskeleton: the cytoskeleton-driven cellular functions. *Histol. Histopathol.* 2000. **15:** 539–549.

13 **Yang, H., Lauzon, W. and Lemaire, I.,** Effects of hyperthermia on natural killer cells: inhibition of lytic function and microtubule organization. *Int. J. Hyperth*. 1992. **8:** 87–97.

14 **Shcherbina, A., Bretscher, A., Kenney, D. M., and Remold-O'Donnel, E.,** Moesin, the major ERM protein of lymphocytes and platelets, differ from ezrin in its insensitivity to calpain. FEBS *Lett.* 1999. **443:** 31–36.

15 **Berryman, M., Gary, R. and Bretscher, A.,** Ezrin oligomers are major cytoskeletal components of placental microvilli: a proposal for their involvement in cortical morphogenesis. *J. Cell Biol.* 1995. **131:** 1231–1242.

16 **Roumier, A., Olivo-Marin, J. C., Arpin, M., Michel, F., Martin, M., Mangeat, P., Acuto, O., Dautry-Varsat, A. and Alcover, A.,** The membrane-microfilament linker ezrin is involved in the formation of the immunological synapse and in T cell activation. *Immunity* 2001. **15** :715–728.

17 **Melder, R. J., Walker, E., Herberman, R. B., Whiteside, T. L.** Adhesion characteristics of human interleukin 2-activated natural killer cells. *Cell. Immunol*. 1991 **132:** 177–192.

18 **Tsukita, S., Yonemura, S., and Tsukita, S.,** ERM (ezrin/radixin/moesin) family: from cytoskeleton to signal transduction. *Curr. Opin. Cell Biol.* 1997. **9:** 70–75

19 **Castelo, L. and Jay, D. G.,** Radixin is involved in lamellipodial stability during nerve growth cone motility. *Mol. Biol. Cell* 1999.**10:** 1511–1520.

20 **Berryman, M., Frank, Z. and Bretscher, A.,** Ezrin is concentrated in the apical microvilli of a wide variety of epithelial cells, whereas moesin is found primarily in endothelial cells. *J. Cell Sci.* 1993. **105:** 1025–1043.

21 **Schwartz-Albiez, R., Merling, A. H., Spring, P., Moller P. and Koretz, K.,** Differential expression of the microspike-associated protein moesin in human tissues. *Eur. J.Cell Biol.* 1995. **67:** 189–198.

22 **Kaneda, K., Kataoka, M., Kishiye, T., Yamamoto, H. and Wake, K.,** The intracellular distribution of cell organelles in natural killer cells during the cytolysis of bound tumor cells, with special reference to the rod-cored vesicles. *Arch. Histol. Cytol.* 1991. **54**: 69–79.

23 **Griffiths, G. M. and Argon, Y.,** Structure and biogenesis of lytic granules. *Curr. Top. Microbiol. Immunol.* 1995. **198:** 39–58.

24 **Bossi, G. and Griffiths, G. M.,** Degranulation plays an essential part in regulating cell surface expression of Fas ligand in T cells and natural killer cells. *Nat. Med.* 1999. **5:** 90–96.

25 **Ramoni, C., Spadaro, F., Menegon, M. and Podo, F.,** Cellular localization and functional role of phosphatidylcholine-specific phospholipase C in NK cells. *J. Immunol.* 2001.**167:** 2642–2650.

26 **Carpen, O.,** The role of microtubules in human natural killer cell-mediated cytotoxicity. Cell *Immunol*. 1987. **106:** 376–86.

27 **Defacque, H., Egeberg, M., Habermann, A., Diakonova, M., Roy, C., Mangeat, P., Voelter, W., Marriott, G., Pfannstiel, J., Faulstich, H. and Griffiths, G.,** Involvement of ezrin/moesin in de novo actin assembly on phagosomal membranes. *EMBO J.* 2000. **19:** 199–212.

28 **Dell'Angelica, E. C., Mullins, C., Caplan, S. and Bonifacino, J. S.** Lysosome-related organelles. *FASEB J*. 2000. **14:** 1265–1278.

29 **Perussia, B., Ramoni, C., Anegon, I., Cuturi, M.C., Faust, J. and Trinchieri, G.,** Preferential proliferation of natural killer cells among peripheral blood mononuclear cells co-cultured with B lymphoblastoid cell lines. *Natural Imm. Cell Growth Reg.* 1987. **6:** 171–188.

Correspondence: Stefano Fais, Laboratory of Immunology, Tumor Immunology Section, Istituto Superiore di Sanità, Viale Regina Elena, 299, I-00161 Rome, Italy
Fax: +39-06-4938-7115
e-mail: fais@iss.it