

Triggering through CD16 or Phorbol Esters Enhances Adhesion of NK Cells to Laminin via Very Late Antigen 6

By Angela Gismondi,* Fabrizio Mainiero,* Stefania Morrone,* Gabriella Palmieri,*[†] Mario Piccoli,* Luigi Frati,* and Angela Santoni*[§]

From the *Department of Experimental Medicine, University of Rome, the [†]Institute for Biomedical Technologies, National Research Council (CNR), and the [§]Laboratory of Pathophysiology, Regina Elena Cancer Institute, 00161 Rome, Italy

Summary

Very late antigens VLA-1, VLA-2, VLA-3, and VLA-6, belonging to the $\beta 1$ subfamily of integrins, have been identified as receptors for different binding domains of laminin (LM). We have detected VLA-6, but not VLA-1 and VLA-2 on a subset (50–70%) of fresh peripheral blood CD3⁻, CD16⁺, CD56⁺ human natural killer (NK) cells by immunofluorimetric and biochemical analysis. Binding assays performed on LM-coated plates showed that 10–15% of NK cells spontaneously adhere to LM, and this adhesion is mediated by VLA-6. Activation of NK cells through CD16 triggering or by phorbol ester results in a rapid increase of adhesion to LM, which is still mediated by VLA-6. The enhanced adhesiveness is not associated with changes in $\beta 1$ LM receptor expression, while it correlates with changes in the phosphorylation status of $\alpha 6$ subunit. The expression of VLA-6 on NK cells and the modulation of its avidity by activating stimuli may be relevant for NK cell migration and tissue location during inflammation or immune response.

NK cells are a heterogeneous population of CD3⁻, CD16⁺, CD56⁺ large granular lymphocytes (LGL)¹ capable of lysing a broad range of neoplastic, normal, and virus-infected cells in a non-MHC-restricted or antibody-dependent manner (antibody-dependent cell-mediated cytotoxicity [ADCC]) (1). CD16 is a low affinity receptor for the Fc fragment of IgG (Fc γ RIII) (2) and represents one of the most important signal transduction structures capable of activating the NK cell functional program (1). NK cells mainly circulate in the peripheral blood, and also can be found under physiologic and inflammatory conditions in several nonlymphoid tissues, including lung interstitium (3), intestinal mucosa (4), and liver (5). This recirculation pattern indicates that they interact with endothelial cells, as well as with several extracellular matrix (ECM) components.

Laminin (LM) is a member of an expanding family of multidomain ECM glycoproteins and one of the major basement membrane components (6). Most cell-surface LM receptors belong to the integrin superfamily (7). In the $\beta 1$ integrin family, four heterodimers have been implicated as LM

receptors: $\alpha 1\beta 1$ (VLA-1); $\alpha 2\beta 1$ (VLA-2); $\alpha 3\beta 1$ (VLA-3); and $\alpha 6\beta 1$ (VLA-6) (8). The more specific LM receptor is VLA-6, which was first identified on platelets (9), but it is also present on monocytes, lymphocytes, and thymocytes (8). Among the LM-binding integrins expressed on leukocytes, only VLA-6 appears to be a functional receptor, and its avidity is rapidly enhanced by short-term cell activation (10, 11).

Previous observations indicate that both human and rodent NK cells express LM, and this molecule may play a role in their cytotoxic functions (12, 13).

Here, we investigated: (a) the expression of LM receptors belonging to the $\beta 1$ family on fresh human NK cells, identifying those which mediate adhesion to LM; (b) the modulation of LM receptor expression and function by stimuli known to trigger NK cells such as CD16 crosslinking or phorbol esters; and (c) the mechanisms that can regulate LM receptor function.

Materials and Methods

Antibodies and Adhesive Proteins. The following mouse mAb were used: anti-CD3 (Leu4), anti-CD16 (Leu11c), anti-CD56 (Leu19), and anti-CD14 (LeuM3), were purchased from Becton Dickinson & Co., (Milpitas, CA); A1A5, against the $\beta 1$ chain of VLA, was kindly provided by Dr. M. Hemler (Dana-Farber Cancer Institute, Boston, MA) (8); J143 and M-KID2 (14), both specific for VLA-3,

¹ Abbreviations used in this paper: ECM, extracellular matrix; LGL, large granular lymphocytes; LM, laminin; LMR, laminin receptors; TPA, 12-O-tetradecanoylphorbol-13-acetate.

were gifts from Dr. C. E. Klein (Department of Dermatology, University of Ulm Oberer Eselsberg, Germany) and Dr. P. G. Natali (Cancer Institute, Regina Elena, Rome, Italy), respectively; anti-VLA-1 was purchased from T Cell Sciences Inc. (Cambridge, MA); anti- $\alpha 2$ was from Telios Pharmaceuticals Inc. (San Diego, CA); and anti-CD16 (B73.1 and 3G8) and anti-MHC I (W6/32), were kindly provided by Dr. G. Trinchieri (Wistar Institute, Philadelphia, PA). The following rat mAb were used: GoH3 (9), specific for the $\alpha 6$, was a gift from Dr. A. Sonnenberg (Laboratory of the Netherlands Red Cross, AD, Amsterdam); and 439-9B (15) (anti- $\beta 1$) was kindly provided by Dr. A. Sacchi (Cancer Institute, Regina Elena). A goat antiserum against the $\beta 1$ subunit was provided by Dr. G. Tarone (University of Turin, Italy) (16).

FITC-conjugated goat F(ab')₂ fragment anti-mouse or anti-rat Ig, rabbit F(ab')₂ fragment anti-goat Ig, and affinity-purified rabbit antiserum against rat, mouse, or goat Ig, were purchased from Cappel Laboratories (Cooper Biomedical Inc., Malvern, PA). LM, both from a murine Engelbreth-Holm-Swazm (EHS) sarcoma (Gibco Laboratories, Grand Island, NY) and from human placenta (Calbiochem Corp., San Diego, CA) was used in adhesion assays.

Purification of Human NK Cells. Highly enriched populations of NK cells from peripheral blood were purified by discontinuous Percoll gradient centrifugation (Pharmacia Fine Chemicals, Uppsala, Sweden) as previously described (17). In several experiments, contaminating T cells were eliminated by panning with anti-CD5 mAb, or by rigorous immunomagnetic negative selection with a cocktail of mAb against CD3 and CD14 antigens. The resulting NK cell populations were more than 90% CD16⁺, CD56⁺, CD3⁻, and CD14⁻, as assessed by cytofluorimetric analysis.

Cell-surface Staining and Cytofluorimetric Analysis. Single- and two-color immunofluorescence were performed as previously described (17). Stained cells were analyzed on a FACScan[®] cytofluorimeter (Becton Dickinson & Co.). Fluorescence intensity is expressed in arbitrary units on a logarithmic scale.

Adhesion Assay. Adhesion assay was performed as previously described (17). Briefly, ⁵¹Cr-labeled purified human NK cells (5 × 10⁴) were allowed to adhere to protein-coated surface for 2 h at 37°C. After removal of unattached cells, adherent cells were solu-

bilized with 1% SDS and bound radioactivity was quantitated in a gamma-counter.

In some experiments, ⁵¹Cr-labeled NK cells were pretreated with 12-O-tetradecanoylphorbol-13-acetate (TPA) (10 ng/ml) or with anti-CD16 mAb or its F(ab')₂ fragments for different times at 37°C. For inhibition of cell adhesion, binding assay was performed in the presence of different concentrations of anti- $\beta 1$, anti- $\alpha 6$, or anti-MHC I antibodies.

¹²⁵I Cell-surface Labeling. Highly purified human NK cells (50 × 10⁶) were surface labeled by lactoperoxidase-catalyzed iodination as previously described (17).

³²P Radiolabeling. Cells were washed in phosphate-free MEM containing 30 mM Hepes and 1% dialyzed FCS and starved for 1 h at 37°C. Cells were resuspended at 50 × 10⁶/ml, incubated with 2 mCi/ml [³²P]orthophosphate for 4 h at 37°C, and then stimulated with the desired agent for the indicated times. Stimulation was terminated by adding ice-cold wash buffer (0.4 mM EDTA-Na₂, 10 mM Na₄P₂O₇, 10 mM NaF, 0.1 mM Na₃VO₄ in PBS, pH 7.4), pelleting cells for 5 min at 500 g, and resuspending them in ice-cold lysis buffer containing 1% β -octylglucoside, 1 mM CaCl₂, 1 mM MgCl₂, 0.1% NaN₃, 1 mM PMSF, 10 U/ml aprotinin, 10 μ g/ml leupeptin, 10 mM NaF, 150 mM NaCl, and 10 mM iodoacetamide for 30 min at 4°C.

Immunoprecipitation and SDS-PAGE Analysis. Lysates from ¹²⁵I or ³²P-radiolabeled cells were immunoprecipitated and analyzed by SDS-PAGE on 7% polyacrylamide gels as previously described (17).

Results

Expression of LM Receptors (LMR) Belonging to the VLA Family by Fresh Human NK Cells. Human peripheral blood NK cells were stained with a panel of mAb directed against $\alpha 1$ (TS 2/7), $\alpha 2$ (P1E6), $\alpha 3$ (J143 or M-KID2), $\alpha 6$ (GoH3), and $\beta 1$ (A1A5), or a polyclonal anti- $\beta 1$ antiserum. The immunofluorimetric analysis shows that LGL, displaying an NK phenotype (CD16⁺, CD56⁺, CD3⁻, CD14⁻), express $\beta 1$ as previously described (17), and a portion of them, the $\alpha 6$

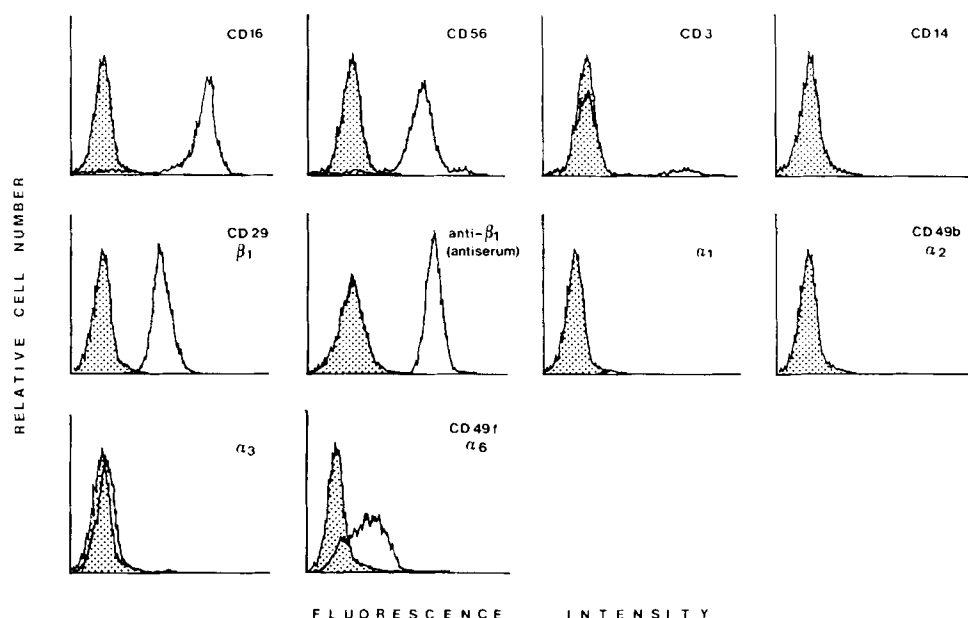


Figure 1. Expression of VLA-6 on fresh human NK cells. Expression of VLA subunits was evaluated by immunofluorescence and flow cytometric analysis. Anti-CD16, anti-CD56, anti-CD3, and anti-CD14 mAb were used to determine the NK phenotype. (Dotted areas) Negative controls.

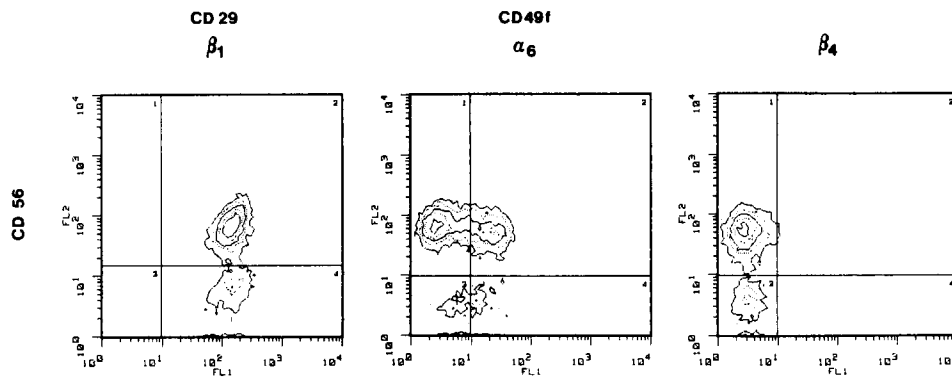


Figure 2. CD56⁺ NK cells express $\alpha6\beta1$, but not $\alpha6\beta4$ integrin. Percoll-enriched human NK cells were stained with anti- $\beta1$, anti- $\alpha6$, or anti- $\beta4$ mAb (green fluorescence, x-axis) and anti-CD56 (Leu 19) mAb (red fluorescence, y-axis).

subunit (50–70%). Although they do not express detectable levels of $\alpha1$ and $\alpha2$, a small amount of $\alpha3$ was found in some donors (Fig. 1). It has been reported that $\alpha6$ subunit can associate with both $\beta1$ and $\beta4$ (18). We therefore analyzed the expression of $\beta4$ subunit on NK cells. As shown in Fig. 2, no detectable levels of $\beta4$ were observed on CD56⁺ NK cells, whereas all of them expressed $\beta1$, and $\alpha6$ was present on a subset. These data indicate that $\alpha6$ is present on NK cell surface as $\alpha6\beta1$ (VLA-6), but not as $\alpha6\beta4$ heterodimer.

Biochemical Characterization of VLA-6 Expressed on Human NK Cells. Cell lysates from ¹²⁵I-radiolabeled highly purified human NK cells were immunoprecipitated with anti- $\beta1$ and anti- $\alpha6$ mAb (Fig. 3). As we have previously shown (17), immunoprecipitation with anti- $\beta1$ mAb resulted in a doublet migrating at 150 and 110 kD under nonreducing conditions, and at 150/130 kD under reducing conditions. Anti- $\alpha6$ mAb immunoprecipitated two proteins migrating at 140 and 110 kD under nonreducing conditions corresponding to the $\alpha6$ and $\beta1$ subunits, respectively. Both proteins migrated at 130 kD under reducing conditions.

Adhesion of NK Cells to LM Is Mediated by VLA-6. To determine whether VLA-6 present on NK cells is a functional

receptor, we analyzed the ability of NK cells to bind to LM-coated surfaces. ⁵¹Cr-labeled human NK cells were incubated on LM-coated plates for 2 h at 37°C in the presence of different doses of GoH3 (anti- $\alpha6$) mAb, or a saturating dose of anti- $\beta1$ antiserum. 10–15% of highly purified NK cells bound specifically to different doses of LM (1–50 μ g/ml) and not to BSA (Fig. 4 A), and this adhesion was completely blocked by anti- $\beta1$ antiserum and, in a dose-dependent manner, by anti- $\alpha6$ mAb. Control antibodies such as anti-MHC I (W6/32) mAb were not inhibitory (Fig. 4 B). These data indicate that NK cells adhere to LM and that this adhesion is mediated by VLA-6.

Activation of NK Cells by CD16-crosslinking or Phorbol Esters Increases Adhesion to LM. We have investigated whether stimuli able to trigger several NK cell functions can modulate their adhesion to LM. ⁵¹Cr-labeled NK cells were treated for different times at 37°C with TPA (10 ng/ml) or,

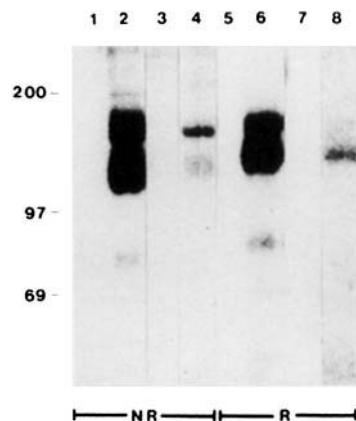


Figure 3. Immunochemical analysis of VLA-6 expressed on human NK cells. ¹²⁵I-labeled human NK cell lysate was immunoprecipitated with control rabbit anti-mouse Ig (lanes 1 and 5), A1A5 (anti- $\beta1$) mAb (lanes 2 and 6), control rabbit anti-rat Ig (lanes 3 and 7), or GoH3 (anti- $\alpha6$) mAb (lanes 4 and 8), and analyzed by SDS-PAGE under nonreducing (NR) and reducing (R) conditions.

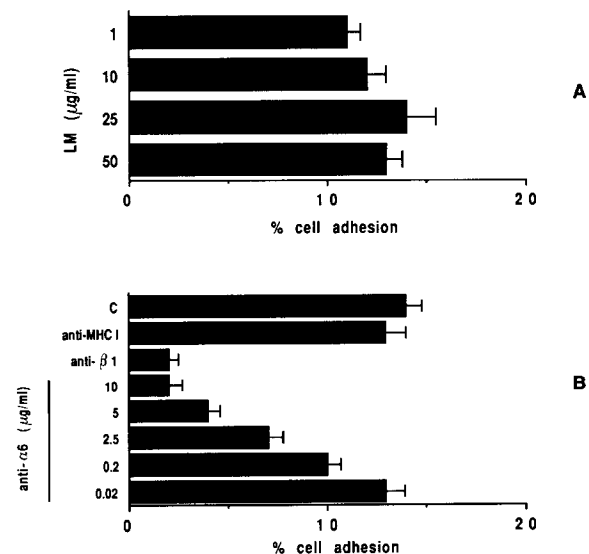


Figure 4. VLA-6 mediates adhesion of human NK cells to LM. (A) adhesion to different doses of LM; (B) adhesion to 10 μ g/ml of LM in the presence of anti- $\beta1$ antiserum or different doses of GoH3 (anti- $\alpha6$) mAb. As control W6/32 (anti-MHC I) mAb was used. Data are presented as the mean of quadruplicate determinations after subtracting the mean of cell adhesion to BSA. Percentage of cell adhesion to BSA was routinely <4%.

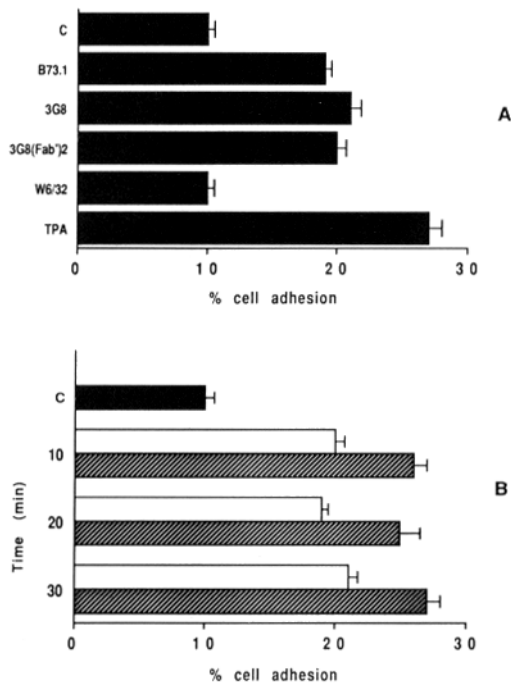


Figure 5. Activation of NK cells by CD16-crosslinking or TPA enhances NK cell binding to LM. (A) ^{51}Cr -labeled human NK cells were treated for 10 min with TPA (10 ng/ml) or with saturating doses of B73.1 or 3G8 mAb or its F(ab)'_2 fragments and assayed for adhesion to LM (10 $\mu\text{g/ml}$) (■). (B) Time course of TPA (▨)- or B73.1 (□)-mediated stimulation of NK cell adhesion to LM; C (■) represents the adhesion of unstimulated cells. Data are presented as the mean of quadruplicate determinations after subtracting the mean of cell adhesion to BSA.

in a more physiologically relevant manner, with anti-CD16 mAb. As shown in Fig. 5 A, activation of NK cells with TPA or by crosslinking of CD16 antigen with saturating doses of B73.1 or 3G8, two mAb directed against different epitopes of this molecule, resulted in enhanced adhesion to LM. Similar results were observed when NK cells were stimulated

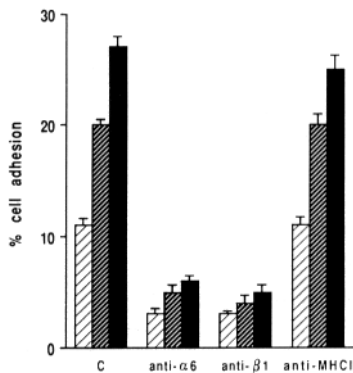


Figure 6. VLA-6 mediates activation-dependent adhesion of NK cells to LM. ^{51}Cr -labeled human NK cells were stimulated for 10 min at 37°C with TPA (10 ng/ml) (■) or B73.1 mAb (▨) and then assayed for adhesion to LM in the presence of anti- $\beta 1$ antiserum, anti- $\alpha 6$ (GoH3), or anti-MHC I (W6/32) mAb; untreated cells (□). Data are presented as the mean of quadruplicate determinations after subtracting the mean of cell adhesion to BSA.

with 3G8 F(ab)'_2 fragments. Enhanced adhesion was already observed 10 min after treatment and remained at the same levels over the stimulation period (30 min) (Fig. 5 B).

VLA-6 Mediates Activation-dependent Adhesion to LM. To investigate whether increased adhesion to LM induced by NK cell activation was mediated by VLA-6, we performed the binding assay in the presence of anti- $\alpha 6$ or anti- $\beta 1$ antibodies. Both anti- $\alpha 6$ and anti- $\beta 1$ antibodies but not control mAb (anti-MHC I) completely blocked adhesion of TPA- or B73.1-stimulated NK cells to LM (Fig. 6). These data indicate that, as for constitutive adhesion to LM, the increased LM binding of stimulated NK cells is exclusively mediated by VLA-6.

Activation-dependent Adhesion to LM Is Not Associated with Changes in the Expression of $\beta 1$ LMR. To understand the mechanisms responsible for the enhanced adhesion of NK cells to LM, we evaluated whether the expression of $\beta 1$ LMR was affected upon NK cell activation. As shown by double-immunofluorescence and cytofluorimetric analysis, treatment with TPA (20 ng/ml for 10 min at 37°C) or anti-CD16 mAb (data not shown) did not affect cell surface expression of $\beta 1$ and $\alpha 6$ subunits (Fig. 7), nor did it induce $\alpha 1$, $\alpha 2$, and $\alpha 3$ subunits (data not shown).

Activation-dependent Adhesion to LM Correlates with Changes in the Phosphorylation Status of $\alpha 6$ Subunit. We have analyzed whether stimulation of NK cells with TPA or anti-CD16 mAb could induce changes in the phosphorylation status of VLA-6. ^{32}P -labeled NK cells were stimulated with TPA or anti-CD16 mAb for different times (5–20 min), and cell extracts were immunoprecipitated with anti- $\alpha 6$ or anti- $\beta 1$ antibodies and analyzed by SDS-PAGE under nonreducing conditions. Both TPA and anti-CD16 (Fig. 8 A), but not anti-CD56 (Fig. 8 B) induced phosphorylation of a 140-kD protein corresponding to the $\alpha 6$ subunit. TPA-induced phos-

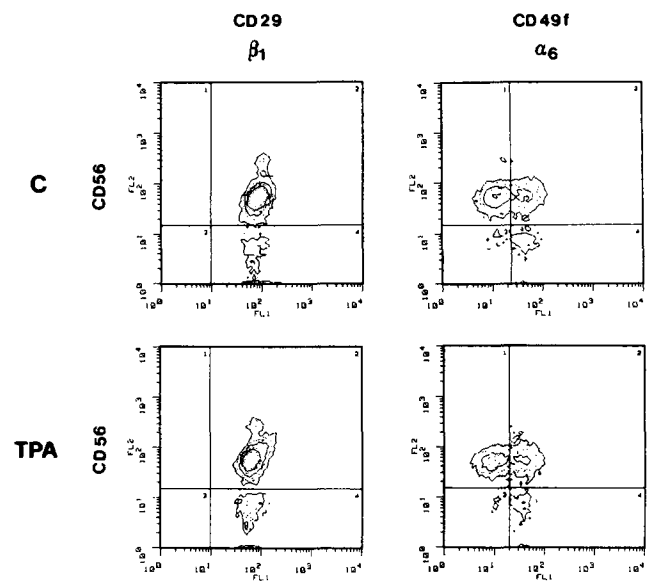


Figure 7. TPA does not affect VLA-6 expression by NK cells. Percoll-enriched human NK cells were stimulated for 10 min at 37°C with TPA (20 ng/ml) and then stained with anti- $\beta 1$ or anti- $\alpha 6$ mAb (green fluorescence, x -axis) and with anti-CD56 (Leu 19) mAb (red fluorescence, y -axis).

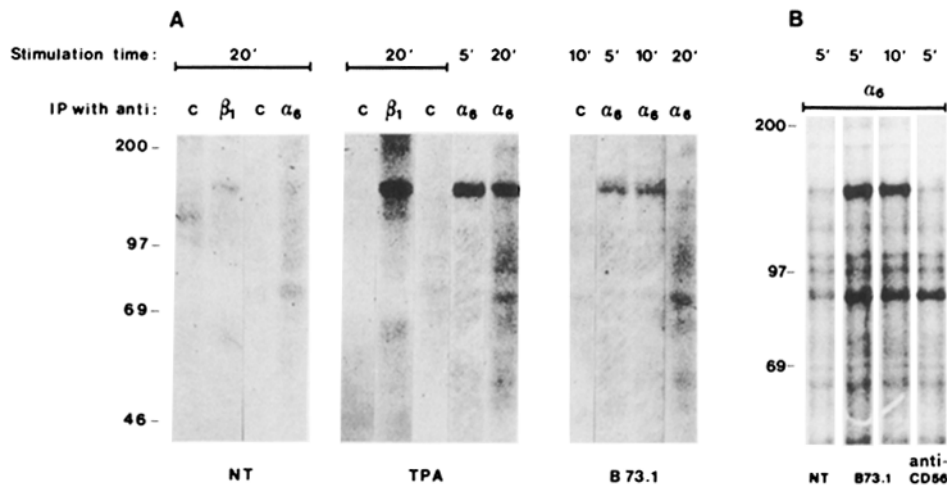


Figure 8. Effect of TPA or anti-CD16 mAb treatment on the phosphorylation status of VLA-6. ^{32}P -labeled NK cells were stimulated with TPA (80 ng/ml) (A) or saturating doses of anti-CD16 (B73.1) (A and B) or anti-CD56 mAb (B) for different times (5–20 min). Cell extracts were immunoprecipitated with control Ig (C), or A1A5 (anti- β 1), or GoH3 (anti- α 6) mAb and analyzed by SDS-PAGE under nonreducing conditions. (NT) Untreated cells.

phorylation was already observed at 5 min and persisted until 20 min after stimulation, whereas that induced by CD16 engagement declined after 10 min. Low levels of α 6 phosphoprotein were present in untreated controls. In these experimental conditions, no phosphorylation of α 6-associated β 1 subunit was observed.

Discussion

The present study provides the first evidence that fresh human peripheral blood NK cells express VLA-6 which mediates their adhesion to LM. It is also shown that triggering of CD16 antigen or TPA enhances this adhesion and is capable of increasing α 6 phosphorylation.

VLA-6 was identified on 50–70% of CD3⁻, CD16⁺, CD56⁺ NK cells by immunofluorimetric and biochemical analysis. α 6 on NK cells was only associated with β 1 but not with β 4, as indicated by the absence of detectable levels of β 4.

The pattern of expression of β 1 LMR on NK cells parallels that observed in resting PBL, which have heterogeneous expression of VLA-6, a small amount of VLA-3, and no detectable levels of VLA-1 and VLA-2 (8, 10).

Our results also show that 10–15% of NK cells spontaneously adhere to LM and that VLA-6 mediates this adhesion. Similarly, low levels of VLA-6-mediated adhesion to LM was observed in fresh memory human CD4⁺ T cells (10). Enhancement of NK cell adhesiveness to LM is induced within a few minutes of stimulation with TPA or CD16 crosslinking, and it remains at the same level over a period of 30 min. This adhesion is still mediated by VLA-6 as shown by mAb blocking experiments.

CD16 triggering-dependent NK cell adhesion to LM is reminiscent of that observed for T lymphocytes stimulated through the TCR/CD3 complex or CD2 antigen (10). Indeed, CD16 on NK cells is a physiologically relevant structure, whose engagement results in kinase activation, increased levels of intracellular calcium, inositol phosphate generation, and activation of lytic machinery and lymphokine produc-

tion (19–21). Our results suggest that VLA-6 and CD16 are coupled by intracellular signaling pathways, i.e., CD16 triggers a cascade of biochemical events or second messenger production that lead to increased VLA-6 adhesive function.

The rapid enhancement of NK cell adhesion to LM upon activation occurs without changes in the levels of cell surface expression of VLA-6 and the other β 1 LMR (data not shown), as previously described for activated T lymphocytes (10), suggesting that a qualitative alteration of integrin is more likely responsible for enhanced adhesiveness. In an attempt to elucidate the biochemical changes occurring after CD16- or TPA-induced cell activation, we observed an increase in the phosphorylation status of α 6. α 6 phosphorylation is already evident after 5 min of stimulation and declines differently depending on the activating agent. Indeed, TPA-induced phosphorylation persists for at least 20 min whereas that induced by CD16 declines after 10 min of stimulation. Constitutive low levels of α 6 phosphorylation were observed, suggesting that NK cell activation may quantitatively rather than qualitatively affect α 6 phosphorylation status. In these experimental conditions, no phosphorylation of β 1 subunit was observed.

The mechanisms involved in the regulation of activation-dependent integrin receptor functions are still unclear. Increased avidity for counter ligands as a result of cell activation is a behavior common to many integrin receptors (7). Nevertheless, the molecular basis of enhanced integrin function is largely unknown. Quantitative changes in cell surface integrin expression appear not to be particularly relevant, as indicated by the failure to demonstrate increased receptor levels accompanying increased binding in many different systems (10, 11, 22) and also in our study. Therefore qualitative changes, i.e., posttranslational modifications of either α and/or β integrin subunits, as well as of other integrin-associated molecules such as cytoskeleton components, are likely to occur. Among the possible posttranslational modifications, phosphorylation appears to be a good candidate as a mechanism regulating integrin receptor functions because of its reversible nature. Cytoplasmic domains of α and β subunits have been shown to be substrates of kinase activities. Neverthe-

less, changes in integrin avidity are not always associated with their phosphorylation, and in some cases they appear to be induced instead by nonproteic components (23, 24).

In regard to β subunits, $\beta 1$ has been shown to be phosphorylated under certain conditions, but the functional significance of this phosphorylation remains unclear (25, 26). With respect to $\beta 2$, serine phosphorylation can be dissociated from phorbol ester-stimulated binding of LFA-1 to intercellular adhesion molecule 1 (ICAM-1) (27).

Phosphorylation of integrin α chains too, has been described. In the $\beta 2$ family, all three α subunits are constitutively phosphorylated in PMN and monocytes (28), but this phosphorylation does not appear to play, at least for α_i , any functional role. In the VLA family, α subunit phosphorylation correlates with the high avidity state of integrin receptor. Phosphorylation of the cytoplasmic domain of $\alpha 6$ subunit and its association to increased adhesion to LM has been observed in our study and in activated murine macrophages (11). $\alpha 6$ phosphorylation suggests an important functional role of α subunit cytoplasmic domain in affinity regulation of integrin receptors. Supporting this idea, recent evidence show that affinity of $\alpha IIb\beta 3$ is modulated by the cytoplasmic domain of αIIb subunit (29). Indeed, its truncation resulted in increased affinity for fibrinogen similar to that observed after exogenous platelet activation. Overall, it can be hypothesized that a sequence in the cytoplasmic domain of α subunit may maintain integrin receptor in a low affinity conformation, likely interacting with an intracellular moiety with "repressor" activity. Modification of this interaction, which can be induced by cytoplasmic domain truncation or phosphorylation, as shown in our study and by Shaw et al. (11), may then result in induction of a high affinity state.

On the basis of the molecular alterations, several mechanisms including cytoskeleton association, interaction with other intracellular moieties, receptor clustering, and exposure of activation epitopes may underlie the increased integrin avidity.

Some of these mechanisms could explain how phosphorylation of $\alpha 6$, induced by CD16 engagement or TPA treatment, results in the enhancement of VLA-6-mediated NK cell adhesion to LM. The lack of correlation between $\alpha 6$ phosphorylation and enhanced adhesion, observed after 20 min of CD16 stimulation, suggests that phosphorylation-induced conformational changes last longer than $\alpha 6$ phosphorylation, and that they are more likely responsible than phosphorylation per se for the high avidity state of the receptor.

Overall, our results suggest that expression of VLA-6 on NK cells appears to be particularly relevant for their migration, tissue localization, and functions. VLA-6-mediated NK cell adhesion to LM is likely to be critical for migration through basement membranes where LM is a major constituent. In this regard, LM has been found to facilitate NK cell migration in response to several chemotactic stimuli (30). The activation-dependent increase in VLA-6-mediated NK cell adhesion to LM may be relevant to temporarily immobilize NK cells at the site of ongoing inflammatory or immune responses, and prevent them from escaping into the circulation.

Whether or not VLA-6/LM adhesion pathway can also be implicated in NK-target cell interaction is unclear. LM has been shown to deliver a costimulatory signal that enhances CD3-induced T lymphocyte proliferation (10), and it has been suggested to facilitate antigen-specific T cell interaction (10). The role of LM-like molecules in NK-mediated cytotoxicity has been reported (13), although it is controversial.

We thank Anna Maria Bressan, Alessandro Procaccini, and Antonio Sabatucci for expert technical assistance, and Sergio Ferraro for photographic assistance. We thank Drs. M. Hemler, G. Tarone, A. Sonnenberg, A. Sacchi, P. G. Natali, C. E. Klein, and G. Trinchieri for kindly providing some of the antibodies used in this study.

This work was partially supported by grants from the Italian Association for Cancer Research, Istituto Superiore di Sanità Italy-USA Program on Therapy of Neoplasias, National Research Council-Clinical Applications of Oncological Research (CNR ACRO).

Address correspondence to Dr. Angela Gismondi, Dipartimento di Medicina Sperimentale, III Cattedra di Patologia Generale, viale Regina Elena 324, 00161 Roma, Italy.

Received for publication 23 March 1992 and in revised form 29 July 1992.

References

1. Trinchieri, G. 1989. Biology of natural killer cells. *Adv. Immunol.* 47:187.
2. Perussia, B., G. Trinchieri, A. Jackson, N.L. Warner, J. Faust, H. Rumpold, D. Kraft, and L.L. Lanier. 1987. The Fc receptor for IgG on human natural killer cells: phenotypic, functional and comparative studies using monoclonal antibodies. *J. Immunol.* 133:180.
3. Prichard, M.G., L.W. Boerth, and J.E. Pennington. 1987. Com-
- partment analysis of resting and activated pulmonary natural killer cells. *Exp. Lung Res.* 12:239.
4. Carman, P.S., P.B. Ernest, K.L. Rosenthal, D.A. Clark, A.B. Befus, and J. Bienenstock. 1986. Intraepithelial leukocytes contain a unique subpopulation of NK-like cytotoxic cells active in the defence of gut epithelium to enteric murine coronavirus. *J. Immunol.* 136:1548.
5. Wiltrout, R.H., B.J. Mathieson, J.E. Talmadge, C.W.

- Reynolds, S. Zhang, R.B. Herberman, and J.R. Ortaldo. 1984. Augmentation of organ-associated NK-activity by biological modifiers: isolation and characterization of large granular lymphocytes from the liver. *J. Exp. Med.* 160:1431.
6. Timpl, R. 1989. Structure and biological activity of basement membrane proteins. *Eur. J. Biochem.* 180:487.
 7. Hynes, R.O. 1992. Integrins: versatility, modulation, and signaling in cell adhesion. *Cell.* 69:11.
 8. Hemler, M.E. 1990. VLA proteins in the integrin family: structures, functions, and their role on leukocytes. *Annu. Rev. Immunol.* 8:365.
 9. Hemler, M.E., C. Crouse, Y. Takada, and A. Sonnenberg. 1988. Multiple very late antigen (VLA) heterodimers on platelets. Evidence for distinct VLA-2, VLA-5 (fibronectin receptor), and VLA-6 structures. *J. Biol. Chem.* 263:7660.
 10. Shimizu, Y., G.A. Van Seventer, K.J. Horgan, and S. Shaw. 1990. Regulated expression and binding of three VLA (beta 1) integrin receptors on T cells. *Nature (Lond.)*. 345:250.
 11. Shaw, L.M., J.M. Messier, and A.M. Mercurio. 1990. The activation dependent adhesion of macrophages to laminin involves cytoskeletal anchoring and phosphorylation of the $\alpha 6\beta 1$ integrin. *J. Cell Biol.* 110:2167.
 12. Morrone, S., S. Scarpa, A. Punturieri, R. Testi, A. Gismondi, G. Santoni, M. Piccoli, L. Frati, A. Modesti, and A. Santoni. 1989. Laminin synthesis by NK cells and modulation of its expression by TPA. *Exp. Cell Res.* 182:543.
 13. Schwarz, R.E., and J.C. Hiserodt. 1988. The expression and functional involvement of laminin-like molecules in non-MHC restricted cytotoxicity by human Leu-19⁺/CD3⁻ natural killer lymphocytes. *J. Immunol.* 141:3318.
 14. Bartolazzi, A., R. Fraioli, G. Tarone, and P.G. Natali. 1991. Generation and characterization of the murine monoclonal antibody M-KID2 to VLA-3 integrin. *Hybridoma.* 10:707.
 15. Falcioni, R., A. Sacchi, J. Resau, and S.J. Kennel. 1988. Monoclonal antibody to human carcinoma-associated protein complex: quantitation in normal and tumor tissue. *Cancer Res.* 48:816.
 16. Conforti, G., A. Zanetti, S. Colella, M. Abbadini, P.C. Marchisio, R. Pytela, F. Giancotti, G. Tarone, L.R. Languino, and E. Dejana. 1989. Interaction of fibronectin with cultured human endothelial cells: characterization of the specific receptor. *Blood.* 73:1576.
 17. Gismondi, A., S. Morrone, M.J. Humphries, M. Piccoli, L. Frati, and A. Santoni. 1991. Human natural killer cells express VLA-4 and VLA-5, which mediate their adhesion to fibronectin. *J. Immunol.* 146:384.
 18. Hemler, M.E., C. Crouse, and A. Sonnenberg. 1989. Association of the VLA $\alpha 6$ subunit with a novel protein: a possible alternative to the common VLA $\beta 1$ subunit on certain cell lines. *J. Biol. Chem.* 264:6529.
 19. O'Shea, J.J., A.M. Weissman, I.C.S. Kennedy, and J.R. Ortaldo. 1991. Engagement of the natural killer cell IgG Fc receptor results in tyrosine phosphorylation of the ζ chain. *Proc. Natl. Acad. Sci. USA.* 88:350.
 20. Cassatella, M., I. Anegon, M.C. Cuturi, P. Grissey, G. Trinchieri, and B. Perussia. 1989. Fc γ R (CD16) interaction with ligand induces Ca⁺⁺ mobilization and phosphoinositide turnover in human natural killer cells. Role of Ca⁺⁺ in Fc γ R (CD16)-induced transcription and expression of lymphokine genes. *J. Exp. Med.* 169:549.
 21. Anegon, I., M.C. Cuturi, G. Trinchieri, and B. Perussia. 1988. Interaction of the Fc receptor (CD16) ligands induces transcription of interleukin 2 receptor (CD25) and lymphokine gene expression of their products in human natural killer cells. *J. Exp. Med.* 167:452.
 22. Dustin, M.L., and T.A. Springer. 1989. T cell receptor cross-linking transiently stimulates adhesiveness through LFA-1. *Nature (Lond.)*. 341:619.
 23. Hermanowski-Vosatka, A., J.A.G. Van Strijp, W.J. Swiggard, and S.D. Wright. 1992. Integrin modulating factor-1: a lipid that alters the function of leukocyte integrins. *Cell.* 68:341.
 24. Conforti, G., A. Zanetti, I. Pasquali-Ronchetti, D. Quagliano, J.P. Neyroz, and E. Dejana. 1990. Modulation of vitronectin receptor binding by membrane lipid composition. *J. Biol. Chem.* 265:4011.
 25. Hirst, R., A. Horwitz, C. Buck, and L. Rohrschneider. 1986. Phosphorylation of the fibronectin receptor complex in cells transformed by oncogenes that encode tyrosine kinases. *Proc. Natl. Acad. Sci. USA.* 83:6470.
 26. Marcantonio, E.E., J. Guan, J.E. Trevithick, and R.O. Hynes. 1990. Mapping of the functional determinants of the integrin $\beta 1$ cytoplasmic domain by site-directed mutagenesis. *Cell Regul.* 1:597.
 27. Hibbs, M.L., S. Jakes, S.A. Stacker, R.W. Wallace, and T.A. Springer. 1991. The cytoplasmic domain of the integrin lymphocyte function-associated antigen 1 β subunit: sites required for binding to intercellular adhesion molecule 1 and the phorbol ester-stimulated phosphorylation site. *J. Exp. Med.* 171:1227.
 28. Buyon, J.P., S.G. Slade, J. Reibman, S.B. Abramson, M.P. Philips, G. Weissman, and R. Winchester. 1990. Constitutive and induced phosphorylation of the α and β -chains of the CD11/CD18 leukocyte integrin family. Relationship to adhesion-dependent functions. *J. Immunol.* 144:191.
 29. O'Toole, T.E., D. Mandelman, J. Forsyth, S.J. Shattil, E.F. Plow, and M.H. Ginsberg. 1991. Modulation of the affinity of integrin α IIb β 3 (GPIIb-IIIa) by the cytoplasmic domain of α IIb. *Science (Wash. DC)*. 254:845.
 30. Somersalo, K., and E. Saksela. 1990. The migration of large granular lymphocytes. *J. Cell. Biochem.* 14(Suppl. A):197.