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Expression and function of α_4/β_7 integrin on human natural killer cells

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SUMMARY

In this report, we have analysed the expression and function of the α_4/β_7 heterodimer in human natural killer (NK) cells. The expression of $\alpha_4\beta_7$ is induced in NK cells upon activation, as the anti-$\alpha_4\beta_7$ ACT-1 monoclonal antibody (mAb) faintly stained a minority of peripheral blood NK cells, whereas it strongly reacted with *in vitro* long-term interleukin-2 (IL-2)-activated NK cells. Incubation with ACT-1 or its $F(ab')_2$ fragments induced a strong homotypic adhesion of NK cells, comparable to that stimulated by the anti-α_4 HP1/7 mAb. Cell–cell interaction induced by the ACT-1 mAb was only prevented by another anti-α_4 mAb (HP2/1) that recognizes a different epitope. In $\alpha_4\beta_7$-mediated cell aggregation, the $\alpha_4\beta_7$ heterodimer was redistributed to intercellular contact sites, thus, suggesting a direct involvement of this integrin in the formation of cell clusters. In NK cells attached to Fibronectin (FN38) or vascular cell adhesion molecule-1 (VCAM-1), both $\alpha_4\beta_7$ and $\alpha_4\beta_1$ integrins were redistributed at the ventral cellular membrane forming discrete contact sites. The ACT-1 mAb only partially blocked NK cell binding to FN38, but in combination with the anti-β_1 mAb LIA1/2, NK cell binding to FN38 was completely inhibited. In contrast, ACT-1 did not modify NK cell adhesion to VCAM-1, thus supporting the theory that the $\alpha_4\beta_7$ binding sites for both ligands appear to be different. Our results indicate that upon IL-2-activation, expression of functional α_4/β_7 integrin is induced on NK cells, potentially participating in their interaction with both extracellular matrix and endothelial cells.

INTRODUCTION

Natural killer (NK) cells constitute a discrete population of $CD3^-CD56^+CD16^+$ peripheral blood lymphocytes involved in non-adaptive immune response mechanisms.[1] In addition to their capacity of mediating non-major histocompatibility complex (MHC)-restricted cytotoxicity against a wide variety of cell types, NK cells can synthesize different cytokines [tumour necrosis factor-α (TNF-α), granulocyte–macrophage colony-stimulating factor (GM-CSF), interferon-γ (IFN-γ) and interleukin-3 (IL-3)][1] and proliferate in response to IL-2, natural killer cell stimulating factor (NKSF)[2] and IL-7.[3] NK cells mediate antibody-dependent cytotoxicity (ADCC) and are considered to participate as a first line of resistance against virus-infected, tumour and allogeneic cells.[4] NK cells migrate to inflammatory foci[5,6] and have been shown to be present in xenograft- and allograft-induced inflammation[7,8] and graft-versus-host disease lesions.[9] As for other leucocytes, the adhesion of NK cells to endothelial cells (EC) and to the extracellular matrix (ECM) is a fundamental process for the recruitment and extravasation.[10]

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Abbreviations: ECM, extracellular matrix; FN, fibronectin; mAb, monoclonal antibody; NK, natural killer; rIL-2, recombinant interleukin-2; VCAM-1, vascular cell adhesion molecule-1; VLA, very late antigen.

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Many of these adhesive functions are mediated by the superfamily of integrins, which is composed by a variety of non-covalently associated heterodimers.[11] Integrins have been grouped into four subfamilies based on the association of distinct β-chains with several α-chains.[11–14] So far, the expression of only β_2 and β_7 integrins seems to be restricted to leucocytes.[15] In some instances, a single α-chain can be associated with more than one β-chain, as in the case of the $\alpha_4\beta_1$ very late antigen-4 (VLA-4) and $\alpha_4\beta_7$ heterodimers. VLA-4 is a receptor for vascular cell adhesion molecule-1 (VCAM-1), and for the CS1/H1 fragment of fibronectin.[16,17] The β_7 integrin subfamily is composed of two members: $\alpha_4\beta_7$, also called $\alpha_4\beta$p,[18] and $\alpha E\beta_7$.[19] The $\alpha_4\beta_7$ heterodimer has also been shown to function as a receptor for fibronectin and VCAM-1 on B[20] and T[21] cells. Moreover, the attachment of murine lymphocytes to Peyer's patch and lamina propria HEV is specifically mediated by $\alpha_4\beta_7$,[22] where it recognizes mucosal addressin cell adhesion molecule-1 (MAdCAM-1), which shares a substantial homology with VCAM-1 and intercellular adhesion molecule-1 (ICAM-1).[23] Recently, it has been reported that there is a heterogeneous expression of $\alpha_4\beta_7$ integrin on $CD4^+$ T cells,[24] and it has been suggested that the transition of $CD4^+$ naive T cells to $CD4^+$ memory T cells is associated with an increase in $\alpha_4\beta_7$ expression. In addition, it has been postulated that $CD4^+$ $CD45RO^{bright}$ T cells with high expression of $\alpha_4\beta_7$ might preferentially recirculate to the gut.[24] NK cells are found mainly in blood and spleen, but are also present in small numbers in non-lymphoid tissues, such as

the liver, lung interstitium and intestinal mucosa.[1] Limited information is available on NK cell adhesion through integrins, whose expression pattern depends on the activation state. In this regard, peripheral blood NK cells express VLA-4 and VLA-5 as fibronectin receptors,[25] and VLA-6 as a laminin receptor,[26] whereas after long-term activation they up-regulate VLA-4 and VLA-5, *de novo* express VLA-1 and VLA-2, while VLA-6 is markedly down-regulated.[27]

In this report, we have studied the expression and function of $\alpha_4\beta_7$ integrin on human IL2-activated NK cells. Our results indicate that the expression of $\alpha_4\beta_7$ is up-regulated upon activation of NK cells, participating in their attachment to fibronectin and VCAM-1. Moreover, stimulation via $\alpha_4\beta_7$ induces homotypic NK cell adhesion comparable to that triggered via $\alpha_4\beta_1$ integrin and both receptors redistributed to intercellular contact sites.

MATERIALS AND METHODS

Monoclonal antibodies (mAb)

Anti-α_1 TS2/7 (IgG1), anti-α_2 TEA1/41 (IgG1), anti-α_4 HP2/1 (IgG1) and HP1/7 (IgG1), anti-α_5 SAM-1 (IgG1), anti-β_1 LIA1/2 (IgG1) and TS2/16 (IgG1) mAb, and anti-CD69 (AIM) TP1/55.3 (IgG1) mAb were obtained in our laboratory and have been already described.[28–30] Anti-VCAM-1 4B9 mAb was kindly provided by Dr J. Harlam (Seattle, WA). Anti-CD7 3A1 (IgG1) mAb was obtained through the American Type Culture Collection (ATCC). ACT-1 (IgG1), which recognizes the $\alpha_4\beta_7$ complex was kindly provided by Dr Lazarovits (Robarts Research Institute, University Hospital and University of Western Ontario, London, Canada) and was previously described.[20,24,31] The P3X63 myeloma supernatant was used as negative control. Anti-CD94 HP3B1 (IgG2a) mAb was obtained in our laboratory.[32]

Cells

Fresh NK cells were purified by treatment of peripheral blood lymphocytes (PBL), depleted of B cells and monocytes, with anti-CD3 mAb plus rabbit complement as described.[32] Long-term IL-2-activated NK cells were obtained essentially as previously described.[33] Briefly, PBL were maintained in culture with irradiated (5 Gy) Daudi-lymphoblastoid cell line (LCL) for 6–8 days in RPMI-1640 supplemented with 10% fetal calf serum (FCS) (complete medium), and a further negative selection, by treatment with anti-CD3 mAb plus rabbit complement, was carried out. CD3− cells were cultured for 5–7 days with 50 IU/ml of recombinant IL-2 (rIL-2), kindly provided by EuroCetus (Madrid, Spain).

FACS analysis

Flow cytometry analyses were performed as described elsewhere.[32] Briefly, 2×10^5 cells, previously treated with aggregated human γ-globulin, to prevent non-specific mAb binding through FcRγIIIA (CD16), were incubated with 100 μl of hybridoma supernatants for 30 min at 4°. After washing with cold phosphate-buffered saline (PBS), cells were stained with goat anti-mouse F(ab′)$_2$-fluorescein isothiocyanate (FITC) (Dakopatts, Glostrup, Denmark). Immunofluorescence was analysed by flow cytometry using a FACScan (Becton Dickinson, Mountain View, CA).

Aggregation assays

NK cells 10^5/well deprived of IL-2 for 24 hr were incubated in triplicate flat-bottomed 96-well microtitre plates (Costar, Cambridge, MA) in a final volume of 100 μl of complete medium supplemented with different mAb (5 μg/ml). In inhibition assays, cells were previously incubated for 20 min at 4° with the blocking mAb. The cells were allowed to settle in a cell incubator at 37° under 5% CO_2 atmosphere for 12 hr. Cell aggregation was assessed by phase contrast microscopy at the specified times and microphotographs of randomly chosen areas were taken.[34]

Adhesion assays

Adhesion of IL-2-activated NK cells to the 38 000 MW fragment of fibronectin, kindly provided by Dr A. García-Pardo (Centro de investigaciones Biológicas, Madrid, Spain)[35] and a soluble form of recombinant VCAM-1, obtained as previously described,[36] was performed as described elsewhere.[20] Briefly, 50 μl/well of FN38 or VCAM-1 in PBS pH 8 (20 μg/ml) were dispensed in 96-well flat-bottomed plates and incubated overnight at 4°. Plates were washed twice with PBS (pH 8) and saturated with 50 μl of bovine serum albumin at 2% (w/v), and further washed with PBS before ^{51}Cr-labelled NK cells were added. For labelling, NK cells were resuspended in complete medium at 10^7 cells/ml and incubated with 100 μCi of sodium ^{51}Chromate (Amersham) at 37° for 1 hr. Cells were washed twice with complete medium and resuspended at the indicated concentration. One hundred microlitres of the cell suspension (10^6 c.p.m./ml) plus 100 μl of indicated mAb (5 μg/ml) were incubated at 4° (for 15 min), dispensed in duplicate (100 μl/well) and incubated for 30 min at 37°. At the end of the incubation, the plates were carefully washed with RPMI-1640 at room temperature. Adherent cells were lysed with 100 μl of PBS plus Triton-X-100 (2%), and radioactivity was measured in a gamma-counter. Results are represented as the percentage of adhesion, referring the mean of duplicate samples to the total input of radioactivity ($> 10^5$ c.p.m./well).

Immunofluorescence studies

Staining of cell aggregates was performed as described.[37,38] Briefly, NK cells were incubated in flat-bottomed plates at 4×10^6 cells/ml in a final volume of 500 μl of complete medium. Monoclonal antibodies or their F(ab′)$_2$ fragments were added in a final concentration of 1 μg/ml and cells were allowed to settle in an incubator at 37° and 5% CO_2 atmosphere. After 15 min, cells were fixed with formaldehyde (3%) in PBS containing 5% sucrose for 10 min at room temperature, and rinsed in TBS (TBS, 50 mM Tris–HCl, 150 mM NaCl, 0·1% NaN_3 pH 7·6). To visualize directly the mAb that induced the aggregation process, cells were stained with a 1:50 dilution of FITC-labelled F(ab′)$_2$ rabbit anti-mouse IgG (Pierce, Rockford, IL). To visualize other membrane proteins, cell aggregation was induced with the F(ab′)$_2$ fragments of the HP1/7 and LIA1/2 mAb and after fixation, the cells were stained with another mAb with a 1:50 dilution of the FITC-conjugated goat-antimouse Fc fragment of IgG (Sigma Chemical Co., St Louis, MO). Cells were observed using a photomicroscope (Nikkon Labophot-2) and photographed on TMAX 400 film (Kodak) processed to 800–1600 ASA with developer (Eastman Kodak Co., Rochester, NY). Staining of adherent cells was performed basically as described.[20] Briefly, glass coverslips

were coated overnight at 4° with FN38 and rVCAM-1 at 20 μg/ml in PBS pH 8. Then, coverslips were rinsed with PBS, saturated with RPMI-1640–1% human serum albumin for 1 hr at 37° and rinsed again. IL-2-activated NK cells were allowed to attach for 2 hr to substrate-coated coverslips. Cells were fixed in 3% of formaldehyde in PBS for 10 min at room temperature. Cells were stained with the primary antibody (ACT-1 or TS2/16) diluted in 50 mM Tris–HCl, 150 mM NaCl, 0·1% NaN_3, pH 7·6 and stained with a 1/40 dilution of a rhodamine isothiocyanate (RITC) goat-anti-mouse IgG (Chemicon, Teruecula, CA). After a final wash the coverslips were rinsed in deionized water and mounted in Mowiol (Calbiochem Behring Corp., San Diego, CA). The samples were observed as described above.

RESULTS AND DISCUSSION

Expression of the $\alpha_4\beta_7$ integrin by human NK cells

It has been shown that the lymphocyte function-associated antigen-1 (LFA-1) and VLA-4 integrins play a crucial role in binding of IL-2-activated NK cells to EC and ECM,[10,39,40] but little is known about the role of the $\alpha_4\beta_7$ integrin. We have comparatively studied by flow cytometry the expression of the α_4-integrins, $\alpha_4\beta_1$ and $\alpha_4\beta_7$, on human NK cells at distinct activation states by using mAb specific for the α_4 chain (HP2/1 and HP1/7), β_1 chain (LIA1/2) and the $\alpha_4\beta_7$ complex (ACT-1). As shown in Fig. 1(a), peripheral blood NK cells (PB NK cells), expressed very low levels of the $\alpha_4\beta_7$ molecule, whereas long term IL-2-activated NK cell populations were brightly stained by the ACT-1 mAb; in contrast, the α_4 chain was detectable regardless of the activation state (Fig. 1b). These data indicate that in PB NK cells, α_4 is mainly associated to β_1-chain forming the VLA-4 heterocomplex, whereas in IL-2-activated NK cells, the α_4 chain is associated to both β_1 and β_7. Incubation of PB NK cells with rIL-2 alone (100 IU/ml) induced an increased expression of the β_7 chain by day 3, reaching the peak at day 10 (Fig. 1b). Moreover, the expression of the $\alpha_4\beta_7$ heterodimer on IL-2-activated NK cells was confirmed by immunoprecipitation experiments (not shown).

To ascertain whether $\alpha_4\beta_1$ and $\alpha_4\beta_7$ expression could define different NK cell subsets, two-colour flow cytometry analyses were performed. All IL-2-activated NK cells coexpressed both $\alpha_4\beta_1$ and $\alpha_4\beta_7$ (not shown). This pattern of distribution is similar to that of IL-2-activated T lymphoblasts (data not shown) and differs from both that of PB B cells that constitutively express $\alpha_4\beta_7$, but are down-regulated upon *in vitro* activation,[20] and that of PB T cells wich include a subpopulation of $CD4^+$ $CD45RO^+$ memory cells with high expression of $\alpha_4\beta_7$.[24]

Binding of activated NK cells to VCAM-1 and fibronectin

Previous reports indicated that long-term IL-2-activated NK cells are able to interact with fibronectin through VLA-4 and VLA-5[25] and with activated EC through LFA-1 and VLA-4.[10,39,40] Moreover, it has been reported that NK cells transmigrate across resting or activated EC monolayers more efficiently than T cells.[39] Thus, we explored the ability of IL-2 activated NK cells to interact with a 38 000 MW FN fragment (FN38) containing the CS1/H1 domain, and with a recombinant form of VCAM-1; the role played by $\alpha_4\beta_7$ and $\alpha_4\beta_1$ heterodimers in these processes was examined comparatively. Blocking experiments showed that anti-α_4 HP2/1 mAb, was able to abrogate completely NK-cell interaction with FN38 (Fig. 2a), indicating that the binding is exclusively mediated by α_4-integrins. However, both anti-β_1 LIA1/2 mAb, which has been reported to block all the adhesive functions mediated by β_1-integrins,[41] and anti-$\alpha_4\beta_7$ ACT-1, were consistently less effective than anti-α_4 HP2/1 mAb in preventing NK-cell interaction to FN38. A complete inhibition of NK-cell adhesion to FN38 was only obtained when cells were

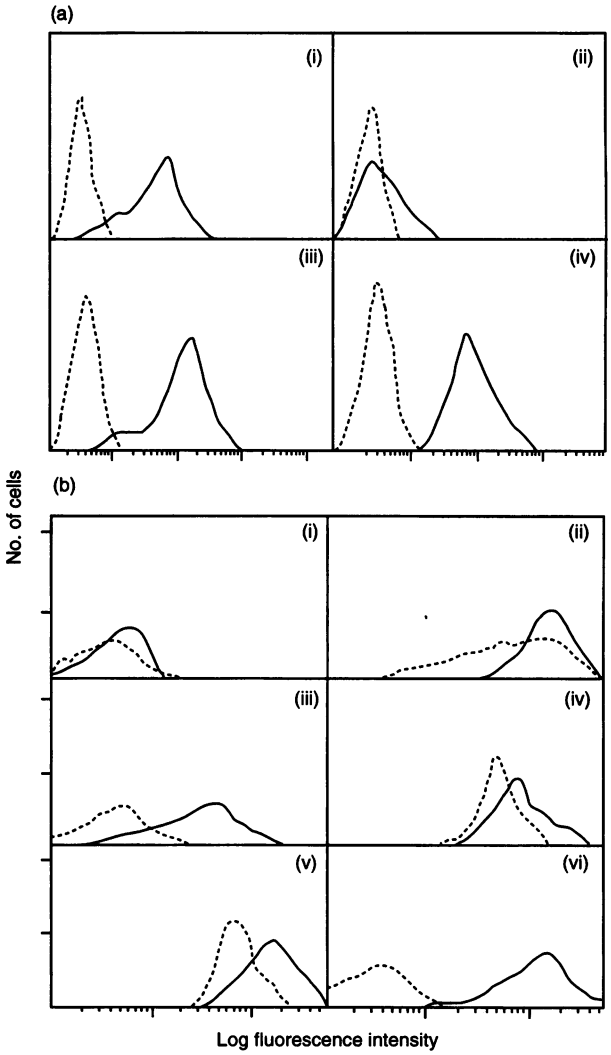

Figure 1. (a) Expression of $\alpha_4\beta_7$ on human NK cells. Imunofluorescence flow cytometry analysis was performed on freshly isolated NK cells (i and ii) or long-term IL-2-activated NK cells (iii and iv) as described in the Materials and Methods. Cells were labelled with anti-CD94 HP3B1 (i and iii) and anti-$\alpha_4\beta_7$ ACT-1 (ii and iv) mAb (solid line). As a negative control the P3X63 myeloma supernatant (dotted line) was used. (b) Imunofluorescence flow cytometry analysis was performed on freshly isolated NK cells either cultured, for 10 days with complete medium (dotted line) or in the presence of 100 U/ml of IL2 (solid line). Cells were labelled with P3X63 (i), anti-CD94 HP3B1 (ii), anti-$\alpha_4\beta_7$ ACT-1 (iii), anti-α_4 (VLA-4) HP1/7 (iv), anti-β_1 LIA1/2 (v) and anti-CD69 TP1/55 (vi) mAb.

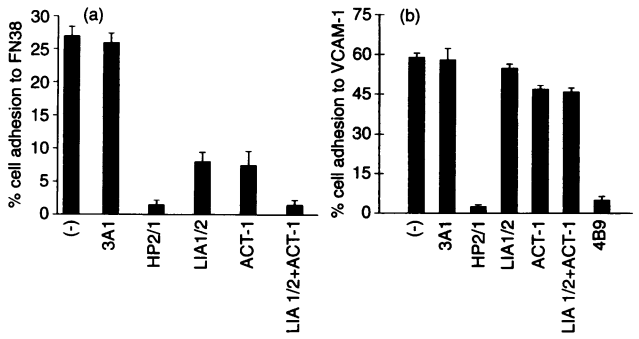

Figure 2. Role of $\alpha_4\beta_7$ and $\alpha_4\beta_1$ integrins in NK-cell interaction with fibronectin and VCAM-1. NK cell binding assays to plates coated with a 38 000 MW proteolytic fragment of FN (a) and a recombinant form of VCAM-1 (b) were performed as described in the Material and Methods. Anti-CD7 3A1, anti-α_4 HP2/1, anti-β_1 LIA1/2 and anti-VCAM-1 4B9 mAb were used at 5 μg/ml, The anti-$\alpha_4\beta_7$ Act-1 mAb was used at 1/500 ascites dilution. The non-specific adhesion to HSA (human serum albumin) was always under 5%. Data represent the average of three different experiments.

simultaneously incubated with anti-β_1 and ACT-1 mAb, thus indicating that the epitope recognized by the ACT-1 mAb on the $\alpha_4\beta_7$ heterodimer may be involved in NK-cell attachment to FN38 (Fig. 2a). It has been previously reported that in PB B cells, which also coexpress $\alpha_4\beta_7$ and $\alpha_4\beta_1$, mAb against β_1 partially blocked cell adhesion to FN38, while mAb against $\alpha_4\beta_7$ have not effect; however, the preincubation with both anti-β_1 and anti-$\alpha_4\beta_7$ totally blocked cell adhesion to FN38.[20] On the other hand, anti-α_4 HP2/1 completely abrogated NK-cell adhesion to VCAM-1 (comparably to the effect of the anti-VCAM-1 4B9 mAb), ruling out that other integrins could be involved in cell binding to this ligand. Remarkably, the addition of anti-β_1 LIA1/2 and anti-$\alpha_4\beta_7$ ACT-1, either alone or in combination, did not significantly modify the attachment to VCAM-1 (Fig. 2b). These results indicate that although both integrins $\alpha_4\beta_7$ and $\alpha_4\beta_1$ are involved in the interaction with VCAM-1, the epitope recognized on the $\alpha_4\beta_7$ complex by ACT-1 is implicated in the binding to FN, and does not account for the interaction with VCAM-1. On the other hand, binding of NK cells to VCAM-1 was unaffected by preincubation of the cells with the anti-β_1 LIA1/2 blocking mAb and/or with anti-$\alpha_4\beta_7$. This is in contrast to PB B cells where a partial inhibitory effect was observed upon preincubation with the anti-β_1 LIA1/2 mAb.[20] A previous report by Allavena *et al.*[39] described an

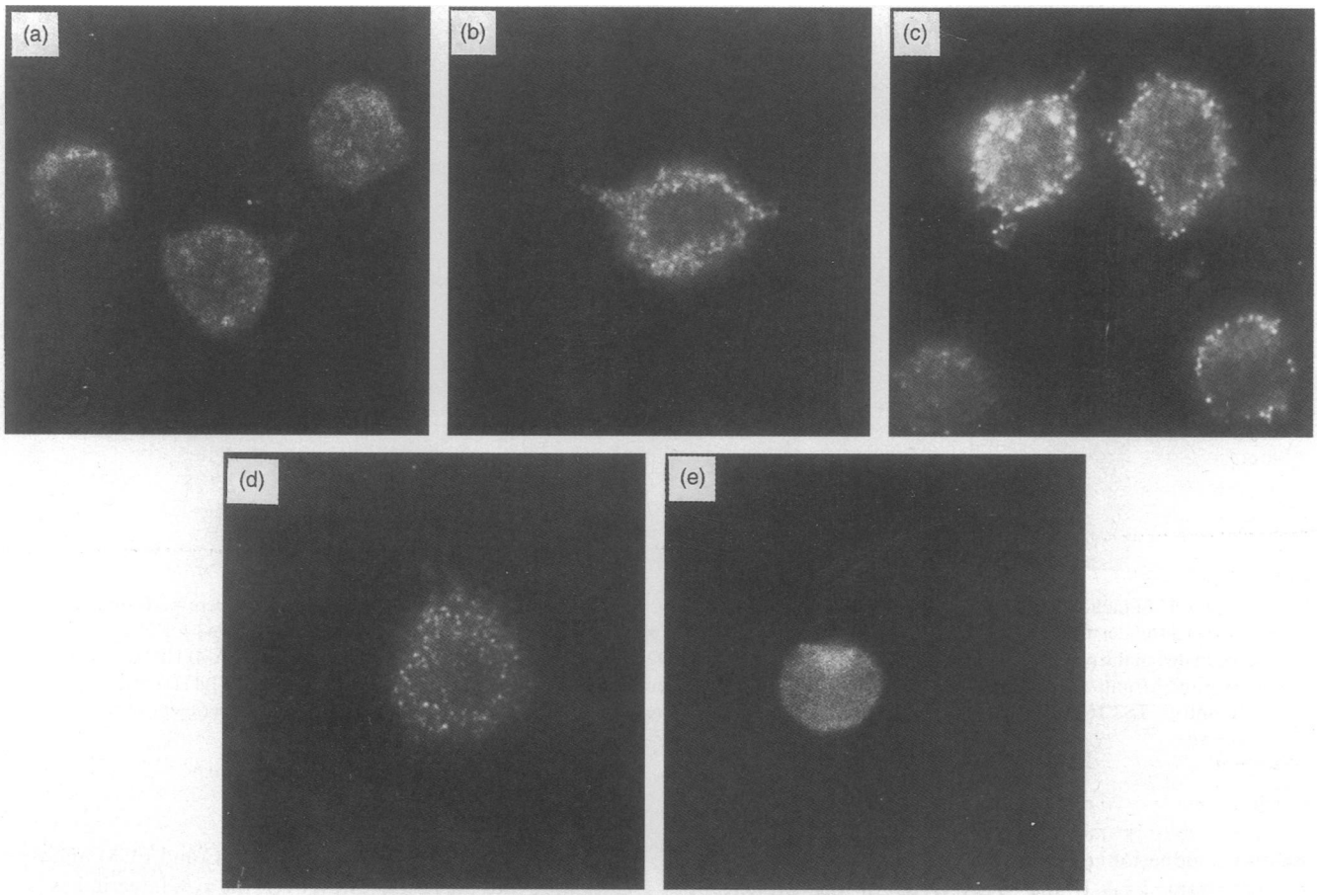

Figure 3. Immunofluorescence analysis of the distribution of $\alpha_4\beta_7$ and $\alpha_4\beta_1$ integrins upon interaction of IL-2-activated NK cells with FN38 or VCAM-1. IL-2-activated NK cells were placed on FN38 (a, b), VCAM-1 (c, d) and BSA (e)-coated surfaces. Cells were stained with anti-$\alpha_4\beta_7$ ACT-1 mAb (a, c and e) and anti-β_1 TS 2/16 mAb (b, d). Experiments were performed as described in the Materials and Methods.

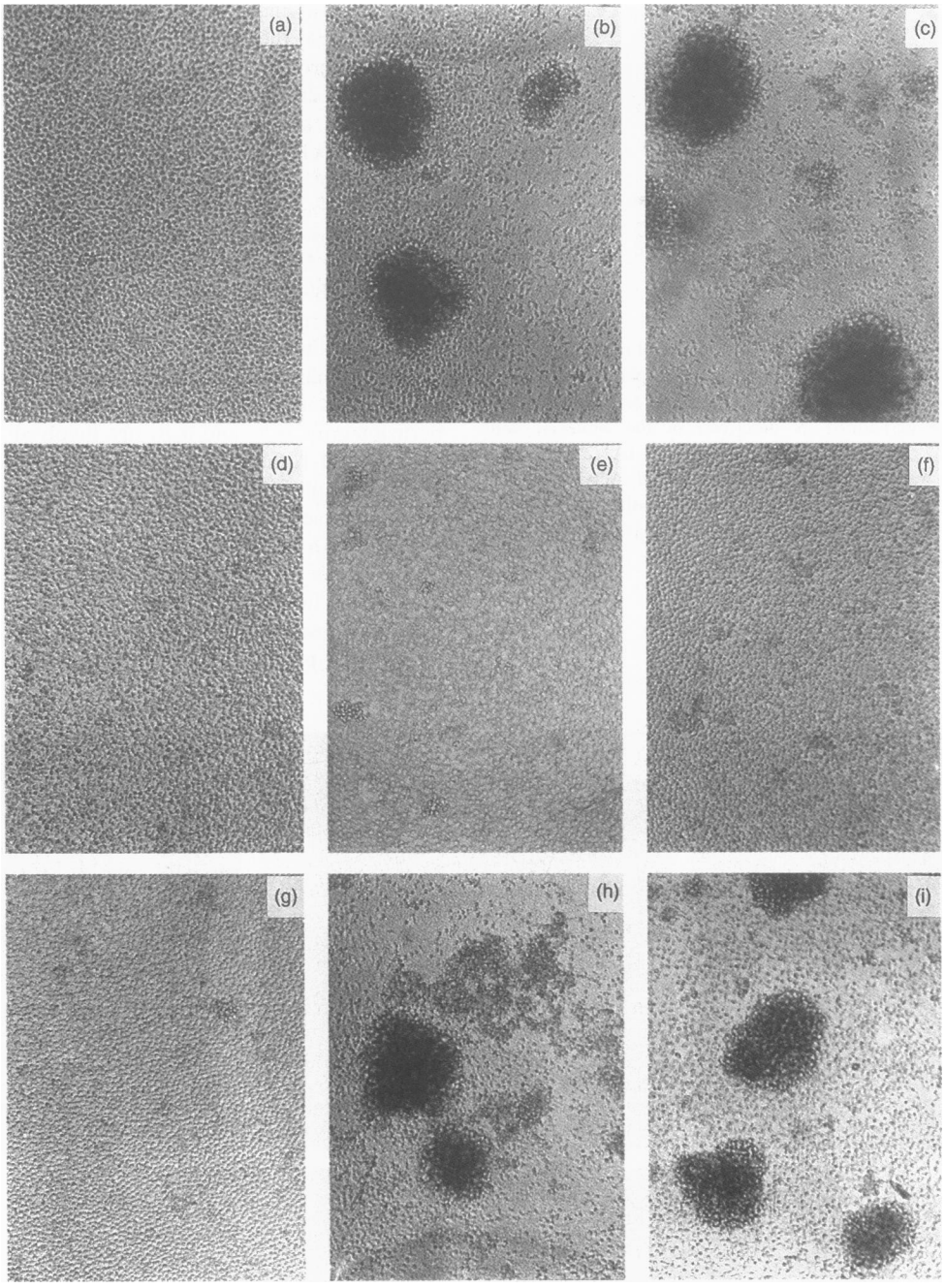

Figure 4. Induction of homotypic cell adhesion of activated NK cells by α_4-integrins. The aggregation assays were performed as described under the Materials and Methods and the cells were photographed at 12 hr. NK cells, deprived of IL-2 for 24 hr were incubated in the presence of the following mAb (5 μg/ml): anti-CD7 3A1 (IgG1) as negative control (a), anti-α_4 (VLA-4) HP1/7 (IgG1) (b, e and h), anti-$\alpha_4\beta_7$ Act-1 (IgG1) (c, f and i). In parallel, cells were preincubated with the blocking anti-α_4 HP2/1 (IgG1) (d, e and f) or anti-β_1 TS2/16 (IgG1) (g, h and i) mAb for 20 min at 4° before adding the inducer mAb. A representative experiment of four is shown.

unknown adhesion pathway to activated EC in NK cells distinct from LFA-1 and VLA-4, as in the presence of saturating concentrations of appropriate anti-CD18 and anti-VLA-4 mAb (± anti-ELAM-1), a proportion of IL-2-activated NK cells clearly remained attached to activated EC. From these results we can conclude that $\alpha_4\beta_7$ may play a pivotal role in the interaction of activated NK cells with EC and ECM and their migration into the tissues. Moreover, the $\alpha_4\beta_7$ integrin has been described to interact with MAdCAM-1 acting as homing receptor, thus allowing activated NK cells to recirculate to the Peyer's patch or lamina propria. Accordingly, the presence of NK cells in the intestinal mucosa has been described.[1]

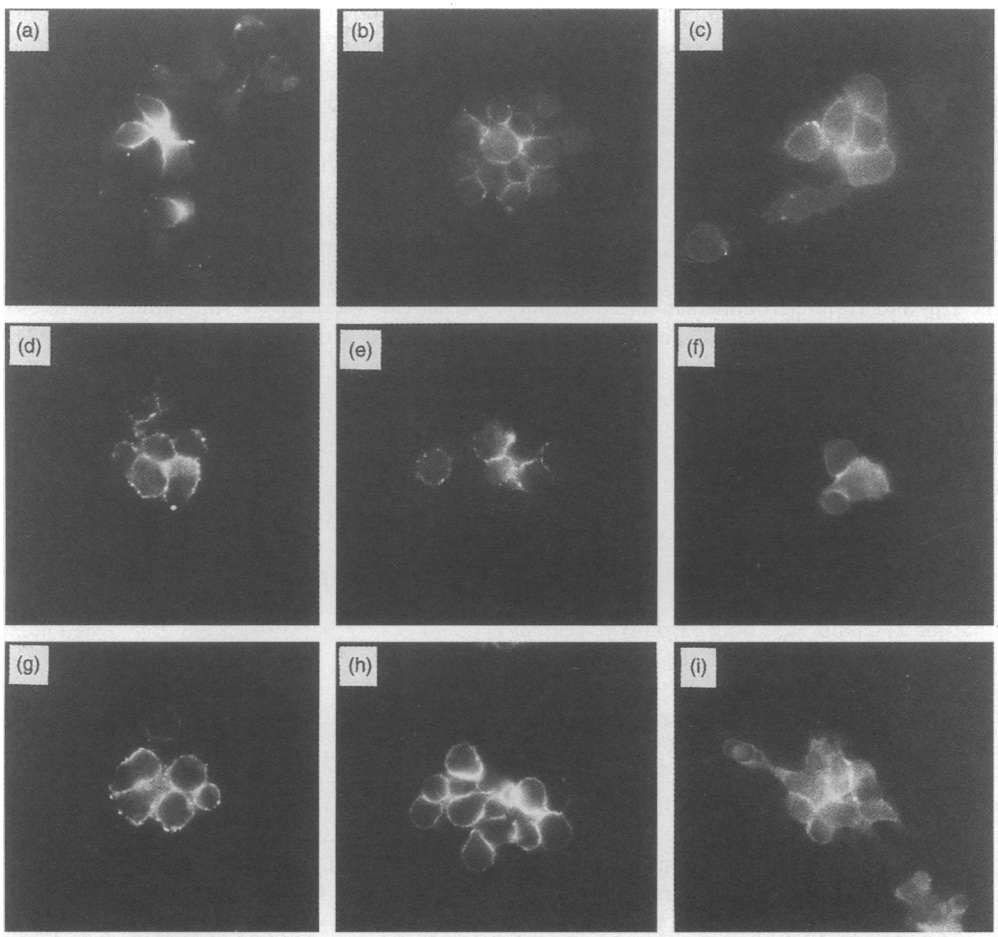

Figure 5. Localization of $\alpha_4\beta_7$, α_4 and β_1 integrins in homotypic cell aggregates. NK cells, deprived of IL-2 for 24 hr, were incubated in the presence of 1 μg/ml of anti-$\alpha_4\beta_7$ ACT-1 (IgG1) (a, d, g), anti-α_4 HP 1/7 (IgG1) (b, e, h) or anti-β_1 LIA 1/2 (IgG1) (c, f, i) for 15 min. Then, cells were fixed with paraformaldehyde and the distribution of anti-$\alpha_4\beta_7$ (a, b, c), anti-α_4 (d, e, h) and anti-β_1 (g, h, i) mAb bound to cells during the aggregation process were detected as described in the Materials and Methods.

Redistribution of $\alpha_4\beta_7$ and $\alpha_4\beta_1$ integrins in discrete clustering sites during NK cell adhesion to fibronectin and VCAM-1

Cell adhesion to ECM proteins induces the reorganization of integrins at the cellular membrane forming discrete contact sites, named focal adhesions, where the integrins colocalize with both the ligand and the cytoskeleton.[42] These multimolecular complexes play an important role in modulating cell-adhesion and inducing cell shape changes involved in cell spreading and locomotion.[43] The α-chains display different amino acid sequences on the cytoplasmic domains whereas the β-chains are more conserved.[15,44,45] Experiments with chimeric integrin receptors, or site directed mutagenesis of α and β subunits, have indicated that the β-cytoplasmic domains are necessary and sufficient to localize integrins to focal adhesions.[46] Therefore, we analysed whether $\alpha_4\beta_7$ and $\alpha_4\beta_1$ showed a different redistribution pattern after NK-cell adhesion to FN38 and VCAM-1 coated surfaces. As shown in Fig. 3, when activated NK cells bound to FN38 (Fig. 3 a and b) or VCAM-1 (Fig. 3c and d) both $\alpha_4\beta_7$ and $\alpha_4\beta_1$ integrins displayed a similar pattern of redistribution in discrete areas at the cell membranes reminiscent of focal adhesions.[42] However, when the cells were plated on bovine serum albumin (BSA) (Fig. 3 e), the distribution of $\alpha_4\beta_7$ was uniform. Despite a different β-chain associated to the α_4 subunit, the relocalization was very similar. This is consistent with the role assigned in this process to a nine-amino acid motif on the cytoplasmic tail of the β_1-chain, seven of which are conserved in the structure of the β_7-chain.[46]

$\alpha_4\beta_7$ and $\alpha_4\beta_1$ are involved in homotypic interactions on human NK cells

β_1 and β_2 integrins have been involved in homotypic cell adhesion of T and B cells.[28,47–49] In NK cells, only LFA-1, a member of the β_2 family of integrins, has been studied in this process.[34,50] We therefore explored the role in this phenomenon of $\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrins. Incubation of activated NK cells with anti-$\alpha_4\beta_7$ ACT-1 mAb or its F(ab')$_2$ fragments strongly induced homotypic NK-cell clustering (Fig. 4c), similar to that induced by anti-α_4 HP1/7 mAb in T cells;[28] in fact, this mAb also induced a strong homotypic NK cell clustering (Fig. 4b). $\alpha_4\beta_7$-mediated cell aggregation was abrogated by preincubating the cells with the anti-α_4 HP2/1 mAb (Fig. 4f) and was

unaffected by mAb directed against other adhesion structures, such as anti-β_1 TS2/16 (Fig. 4i), anti-LFA-1, anti-ICAM-1 anti-CD2 or LFA-3 (not shown). Both, ACT-1 and HP1/7-induced aggregation involved an active process, as it was blocked at 4° or after incubation in the presence of 5 mM deoxyglucose (not shown). Moreover, an intact cytoskeleton was required as demonstrated by the inhibitory effect of incubation in 1 mM cytochalasine D (not shown). These results strongly suggest an active role of the $\alpha_4\beta_7$ and $\alpha_4\beta_1$ heterodimers in homotypic NK-cell adhesion. To study the involvement of the $\alpha_4\beta_7$ and $\alpha_4\beta_1$ heterodimers in the clustering process, we analysed the membrane redistribution of these heterodimers after the induction of aggregation with anti-$\alpha_4\beta_7$ (Fig. 5 a, d, g), anti-α_4 (Fig. 5 b, e, h) and anti-β_1 (Fig. 5 c, f, i) mAb. After cell fixation, we visualized $\alpha_4\beta_7$ (Fig. 5 a, b, c), α_4 (Fig. 5 d, e, f) and β_1 (Fig. 5 g, h, i) with FITC-labelled anti-mouse IgG or RITC goat-antimouse IgG Fc. We observed that $\alpha_4\beta_7$ heterodimers localized at cell–cell contacts mainly when the clustering was induced by anti-$\alpha_4\beta_7$ (Fig. 5a) or anti-α_4 (Fig. 5b), but not with anti-β_1 (Fig. 5c). When we visualized the α_4-chain on aggregates induced by anti-$\alpha_4\beta_7$ mAb (Fig. 5 d), we observed that the α_4-chains were only partially relocated at the cell–cell contacts. Nevertheless when the inducer mAb was anti-α_4, the localization of the α_4-chains was preferentially at the cell–cell boundaries (Fig. 5 e). By contrast, β_1-chains were partially relocated to cell–cell contacts only on aggregates induced by anti-α_4 and anti-β_1 (Fig. 5 h, i, respectively) and not on aggregates induced by anti-$\alpha_4\beta_7$ mAb. In all experiments, anti-CD7 3A1 mAb was used as negative control, no reorganization of CD7 was observed (not shown).

On NK cells, different stimuli have been involved in the induction of homotypic cell adhesion, including phorbol esters or mAb against different surface molecules, such as CD94, CD16,[51] and LFA-1.[34] Monoclonal antibodies directed to certain epitopes of the α_4-chain, are able to induce homotypic cell adhesion in T cells mediated, at least in part, by VLA-4 itself.[28] This is the first evidence that, in NK cells, the $\alpha_4\beta_7$ and $\alpha_4\beta_1$ heterodimers are directly involved in inducing homotypic cell adhesion as concluded by the observation that ACT-1 and HP1/7 mAb actively up-regulate homotypic cell aggregation and the membrane relocalization of the heterodimers on cell aggregates. This cell clustering appears to be mediated by $\alpha_4\beta_7$ and $\alpha_4\beta_1$ molecules themselves being independent of LFA-1 and other adhesion structures. As occurs with $\alpha_4\beta_1$, the counterpart ligand of $\alpha_4\beta_7$ has recently been addressed;[52] it has been found that the α_4-chain and a 34 000 MW proteolytic fragment of the α_4-chain, can bind lymphocytes in the presence of $\alpha_4\beta_1$ and $\alpha_4\beta_7$ at the cell surface. An amino acid motif LDV in the α_4 subunit and the 34 000 MW fragment mediates the binding and appears to be involved in the α_4-dependent homotypic aggregation.[52] The precise mechanism of this phenomenon remains to be elucidated, although triggering of signal transduction induced by some mAb inducing aggregation has also been implicated.[53,54]

So far, there is limited information about the putative role of β_1-integrins as accessory molecules in cellular cytotoxicity and cytokine production mediated by NK cells.[55] It has been described that under certains conditions fibronectin and specific mAb for VLA-4 and VLA-5 are able to up-regulate NK cell-mediated cytotoxicity against certain NK-susceptible lymphoid cell lines.[55] When we examined the possible involvement of $\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrins in NK-cell function; we found that soluble mAb directed against α_4 (HP1/7, HP2/1), β_1 (TS 2/16 and LIA 1/2) and $\alpha_4\beta_7$ (ACT-1) did significantly modify neither their cytotoxic activity against tumour cell lines, nor their cytokine (TNF-α) secretion.

The present report suggests a complementary role for the $\alpha_4\beta_7$ and $\alpha_4\beta_1$ heterodimers in the regulation of adhesive properties of activated NK cells potentially involved in their interaction with both ECM and EC, regulating the transmigration through the endothelial barrier to the tissues. Moreover, the expression of $\alpha_4\beta_7$ may potentially confer to activated NK cells the capacity of migrating to intestinal mucosa by interacting with the human homologue of MAdCAM-1.

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