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# Activation of natural killer cells by the mAb YTA-1 that recognizes leukocyte function-associated antigen-1

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*Key words*: LFA-1, natural killer, YT cell line


## Abstract

**The mAb YTA-1, which brightly stains $CD3^-CD16^+$ large granular lymphocytes (LGL)/natural killer (NK) cells and $CD8^+$ T cells by immunofluorescence, is specific for leukocyte function-associated antigen (LFA)-1. Some mAbs recognizing the LFA-1α chain (CD11a) or LFA-1β chain (CD18) inhibited the binding of YTA-1 to peripheral blood mononuclear cells. YTA-1 mAb could be chemically cross-linked to 170 and 96 kDa molecules, whose molecular weights correspond to those of LFA-1α and β respectively. YTA-1 bound to COS-7 cells co-transfected with CD11a and CD18 cDNAs, but not to untransfected cells. Reactivities of YTA-1 to K562 cells transfected with LFA-1α and β (CD11a/CD18) cDNAs and to CHO cells transfected with Mac-1 (CD11b/CD18) or p150, 95 (CD11c/CD18) cDNAs strongly suggest that YTA-1 recognizes either LFA-1α or an epitope formed by a combination of LFA-1α and β. Treatment of fresh $CD3^-CD16^+$ LGL with YTA-1 augmented cytolytic activity and induced proliferation. $F(ab')_2$ fragments of YTA-1 augmented NK cytotoxicity, indicating that the NK activating signal was transmitted through LFA-1 without involvement of Fcγ receptor III. In contrast, the other mAbs against LFA-1 could not activate NK cells. These results collectively indicate that YTA-1 recognizes a unique epitope of LFA-1, which is involved in activation of fresh NK cells.**


## Introduction

Natural killer (NK) cells are important in the host's early immune response to viral infection and malignant transformation. However, the cell surface structures that mediate activation of NK cells are poorly understood. In an attempt to define the receptor of NK cells for target cells, several kinds of molecules expressed preferentially in NK cells, i.e. NKRP-1, NKG2, Ly49 and NK1.1, have been identified (reviewed in 1). Some, if not all, of these molecules can transmit a signal(s) in NK cells, such as phosphoinositide turnover and an increase in $[Ca^{2+}]_i$ (2,3). Other candidates for NK receptors, such as p58 that binds MHC class I (4); 2B4, a member of the Ig supergene family (5); and the p104-related molecules defined by an anti-idiotype antibody have been documented (6). On the other hand, experiments designed to block NK cytotoxicity by a series of mAbs showed that adhesion molecules such as leukocyte function-associated antigen (LFA)-1 and CD2 are involved in the interaction of NK cells with their targets (7,8). Furthermore, mAbs to CD2 could activate NK cells (9). Despite extensive studies, the molecule(s) responsible for recognition of targets and subsequent activation of NK cells remains to be determined.

We previously established a human large granular lympho-

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cyte (LGL)/NK-like cell line, YT (10), and generated murine mAbs against these cells (11). One of the mAbs, YTA-1, is mitogenic for normal peripheral blood mononuclear cells (PBMC) and down-modulates the expression of the high- and intermediate-affinity IL-2 receptors on the cell surface. However, YTA-1 bound to the MT-1 cell line which did not express IL-2Rβ, indicating that YTA-1 does not react with IL-2Rβ (12). The present work was undertaken to identify YTA-1 antigen. In addition, experiments were carried out to determine whether YTA-1 can activate normal NK cells, since YTA-1 antigen was strongly expressed not only on the YT cell line but also on normal $CD3^-CD16^+$ LGL.

## Methods

### *Cells*

Human PBMC were isolated from normal donors by Ficoll-Hypaque gradient centrifugation. To isolate NK cells, adherent cells in PBMC were depleted using serum-coated plastic dishes, Sephadex G10 and nylon-wool columns. Non-adherent cells were further fractionated on discontinuous seven-step Percoll density gradients, as described (13). The cells in appropriate low-density fractions were depleted of T cells using OTK3 mAb and magnetic beads coated with goat anti-mouse IgG antibody (Dynabeads® M-450; Dynal, Oslo, Norway). More than 90% of the final cell preparation was $CD3^-CD16^+$ LGL. Cells of the human NK-like line, YT (10), were cultured in RPMI 1640 medium (Gibco, Gaithersburg, MD) supplemented with 10% heat-inactivated FCS (Flow, McLean, VA) and antibiotics.

### *Antibodies*

YTA-1, TS1/22 (anti-LFA-1α) (14), TS1/18 (anti-LFA-1β) (14), CBRM1/29 (anti-Mac-1α) (15) and CBRp150, 95/4G1 (anti-p150, 95α) (16) mAbs were purified from ascites of BALB/c mice injected with the respective hybridomas as described previously (12). X63 and MOPC31C are IgG1 mouse myeloma proteins. The $F(ab')_2$ fragments of YTA-1 were prepared by dialyzing purified YTA-1 mAb against 0.1 M acetate (pH 4.2), followed by digestion with pepsin (Sigma, St Louis, MO) at 37°C for 12 h. The $F(ab')_2$ fragments were purified by gel filtration on a Sephacryl S200 column. An anti-IL-2 mAb containing $1\times10^6$ neutralizing activity units/mg protein was obtained from Cosmo-Bio (Tokyo, Japan). Anti-human LFA-1α mAb, SPV-L7 anti-CD11a mAb, an anti-CD18 mAb and 84H10 anti-ICAM-1 mAb were obtained from Becton Dickinson Immunocytometry System (San Jose, CA), Sanbio (AmUden, Holland), Immunotech (Marseilles, France) and Cosmo-Bio respectively. YTA-1 mAb was biotinylated by the use of *N*-hydroxysuccinimidobiotin (Pierce, Rockford, IL) following manufacturer's instruction. The OKT3 hybridoma was obtained from ATCC (Rockville, MD).

### *Immunofluorescence analysis*

Either $1\times10^5$ cell line cells or $5\times10^5$ PBMC were incubated on ice with a saturating amout of mAb, washed and stained with FITC-conjugated goat anti-mouse IgG (Cappel Research Products, Durham, NC). In competition experiments to block the staining with YTA-1, the cells were incubated with saturating concentrations of anti-LFA-1 mAbs on ice for 30 min, washed and further incubated with 20 μl of MOPC31C ascitic fluid. Then the cells were stained with a saturating concentration (100 μg/ml) of biotinylated YTA-1 mAb, washed and incubated with phycoerythrin-conjugated streptavidin (Biomeda). The analysis was performed with a flow cytometer Cytoron (Ortho Diagnostic Systems, Tokyo, Japan).

### *Cell surface labeling, chemical cross-linking and immunoprecipitation*

YT cells ($4.0\times10^7$) were washed extensively with Hanks' balanced salt solution (HBSS), transferred to a glass tube coated with lodogen (Pierce), incubated with 18.5 MBq $Na^{125}I$ at room temperature for 10 min and then washed three times with HBSS containing 10 mM KI. To chemically cross-link YTA-1 mAb to cell surface antigen, $^{125}I$-labeled cells suspended in HBSS ($10^8$ cells/ml) were incubated with 100 μg/ml of YTA-1 or control mAbs on ice for 30 min and dithiobis-succinimidyl-propionate (DSP) (Pierce) was added to a final concentration of 0.8 mM. After incubation at room temperature for 15 min, $1\times10^7$ cells were washed twice with HBSS, solubilized in 1 ml of a lysis buffer (17) containing 10 mM Tris–HCl, pH 7.4, 66 mM EDTA, 1% Nonidet P-40, 0.4% deoxycholate, 1% aprotinin and 1 mM phenylmethylsulfonyl fluoride on ice for 30 min. The extract was centrifuged at 10,000 *g* at 4°C for 10 min, and the complex of YTA-1 mAb and antigen was immunoprecipitated with 10 μg rabbit anti-mouse IgG antibodies (Cappel Research Products) and 20 μl Pansorbin (Calbiochem, San Diego, CA). The immune complexes were washed extensively with 10 mM Tris–HCl, pH 7.4, 150 mM NaCl, 1% Nonidet P-40, 0.4% deoxycholate and 0.1% SDS, and analyzed by SDS–PAGE under reducing conditions.

### *Transfection of LFA-1α and β chain cDNAs*

COS-7 cells were transfected with 10 μg of LFA-1α (18) and/or β (19) cDNAs by electroporation (Gene-Pulser; BioRad, Cambridge, MA) at 400 V, 125 μF in a 0.4 cm cuvette, as previously described (20). Two days later, cells were detached by incubation in PBS containing 2 mM EDTA for 20 min at 37°C and analyzed by flow cytometry. CHO cells transfected with Mac-1 (21), p150, 95 (22) or ICAM-1 (23) cDNAs were maintained in α-MEM supplemented with 10% dialyzed FCS, 16 μM thymidine, 0.10 μM methotrexate, 2 mM glutamine and 50 μg/ml gentamicin (15,24). Prior to immunostaining, cells were detached after a 5 min treatment with HBSS containing 10 mM HEPES, pH 7.3, and 5 mM EDTA. K562 cells were co-transfected by electroporation with cDNAs for LFA-1α, LFA-1β and a plasmid that confers hygromycin resistance (L. Petruzzelli and T. A. Springer, in preparation). Positive clones that expressed high levels of surface LFA-1 were selected by single cell cloning and flow cytometry.

### *Treatment of LGL and cytotoxicity assay*

Purified $CD3^-CD16^+$ LGL or PBMC ($10^6$ cells/ml) were cultured in the presence of varying doses of YTA-1 mAb, or the $F(ab')_2$ fragments of YTA-1, and/or human recombinant IL-2 (Shionogi Pharmaceutical, Osaka, Japan) at 1000 U/ml for 18 h at 37°C in serum-free T medium (Daigo, Osaka, Japan), as described (13,25). In some experiments, greater

than a saturating concentration of anti-IL-2 was added to the culture. Treatment with YTA-1 mAb did not affect the viability of cells recovered. The cells were washed and suspended in complete medium (RPMI 1640 supplemented with 10% FCS). Varying numbers of the effector cells treated as above were incubated with $^{51}$Cr-labeled K562 target cells ($5\times10^3$ cells) for 4 h at 37°C in wells of round-bottom microtiter plates. Samples were then harvested and the radioactivity in the supernatant was determined in an autogamma scintillation counter, as described (13). The percent cytotoxicity was calculated as [(experimental release − spontaneous release)/(maximum release − spontaneous release)]×100. Results are expressed as the mean cytotoxicity ± SD or lytic units (LU) per $10^6$ cells. One LU was defined as the activity to produce 30% lysis of targets.

*Proliferation assay*

Purified CD3⁻ LGL suspended in T medium containing 10% human AB serum (ICN Biochemicals, Costa Mesa, CA) were placed in microtiter plates at $10^5$ cells/well and cultured in the presence of varying doses of YTA-1 mAb or human recombinant IL-2 for 96 h at 37°C. To each well, 37 kBq [$^3$H]thymidine was added for the last 20 h of culture. Cultures were harvested and incorporated radioactivity measured by liquid scintillation as previously described (25). Results are expressed as the mean c.p.m. ± SD.

*Determination of IFN-γ production*

Purified CD3⁻ LGL were placed in round-bottom microtiter plates at $2\times10^5$ cells/well and cultured in the presence of 20 μg/ml of YTA-1 or TS1/22 mAb. After culture for 48 or 96 h, supernatants were harvested and concentrations of IFN-γ were measured using a commercial ELISA (BioSource International, Camarillo, CA).

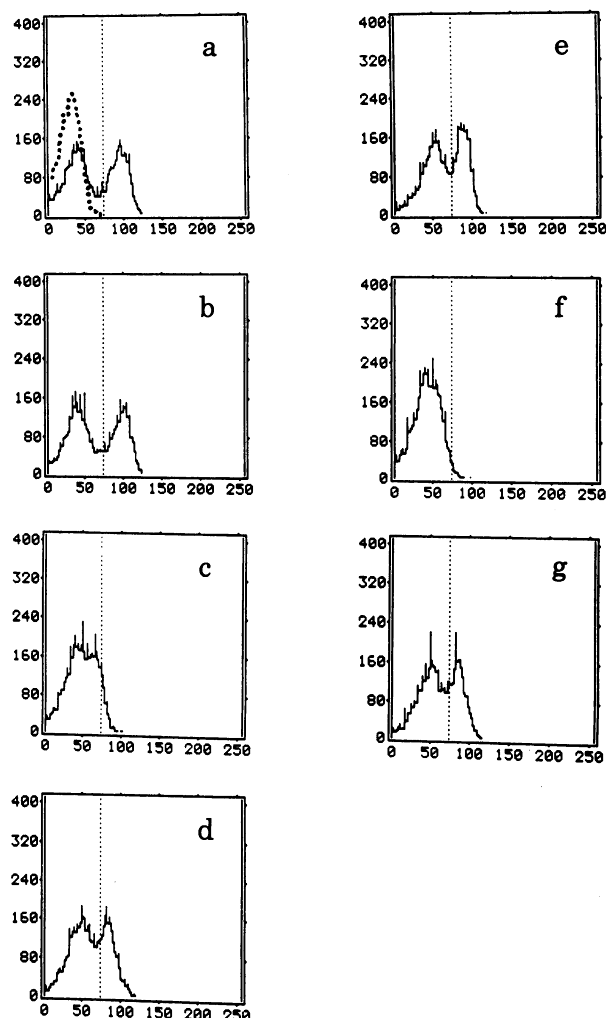


**Fig. 1.** Competition of YTA-1 staining by anti-LFA-1 mAbs. Normal PBMC were preincubated with no mAb (a), anti-CD54 mAb (b), anti-human LFA-1α mAb (Becton Dickinson) (c), TS1/22 anti-LFA-1α mAb (d), SPV-L7 anti-LFA-1α mAb (e), TS1/18 anti-LFA-1β mAb (f) or an anti-LFA-1β mAb (Immunotech) (g), washed, and stained with biotinylated YTA-1 mAb and phycoerythrin–streptavidin. The ordinate and abscissa represent the cell number and the fluorescence intensity on a logarithmic scale respectively. The dashed line in (a) represents the immunofluorescence of PBMC preincubated with excess amount of YTA-1 mAb and stained with biotinylated YTA-1 mAb and phycoerythrin–streptavidin. Staining with MOPC31C IgG showed an identical pattern (not shown). The results were reproduced in three independent experiments.

## Results

*Identification of YTA-1 antigen as LFA-1*

Since YTA-1 antigen is strongly expressed on virtually all CD16⁺ LGL and ~50% of peripheral blood CD3⁺ cells (11), we investigated possible relationship between YTA-1 antigen and LFA-1, which was known to be expressed on these cells. Two-color flow cytometry of normal PBMC revealed that the expression pattern of YTA-1 antigen and LFA-1 was similar, i.e. bright in CD16⁺ cells, bright in ~50% of CD3⁺ cells, dim in CD20⁺ cells, bright in CD8⁺ cells and dim in CD4⁺ cells (data not shown). We therefore determined whether various monoclonal anti-LFA-1 are capable of blocking the binding of YTA-1 to the cells. The biotinylated YTA-1 mAb bound to normal PBMC in a bimodal manner, i.e. dim and bright. Preincubation with anti-LFA-1 mAbs blocked the subsequent staining with YTA-1 to various degrees (Fig. 1). Of five mAbs tested, an anti-human LFA-1α mAb from Becton Dickinson Immunocytometry System and TS1/18 (anti-LFA-1β) most potently blocked YTA-1 staining. In contrast, TS1/22 (anti-LFA-1α), SPV-L7 (anti-LFA-1α) and an anti-LFA-1β mAb from Immunotech did not significantly block YTA-1 binding. Anti-CD54 mAb failed to diminish YTA-1 binding.

Since the flow cytometric data described above suggest that YTA-1 mAb binds to LFA-1, we tried to compare the molecular weights of YTA-1 Ag and LFA-1 by immunoprecipitation. As expected, LFA-1 was immunoprecipitated by TS1/18 from the lysates of surface-labeled YT cells. Under the same conditions, however, we were unable to detect any peptide in the immune complex of YTA-1 (data not shown). Considering the relatively low binding affinity of YTA-1 mAb ($K_d$ = 30–50 nM) as determined by Scatchard analysis (K. Sugie and K. Teshigawara, unpublished observation),

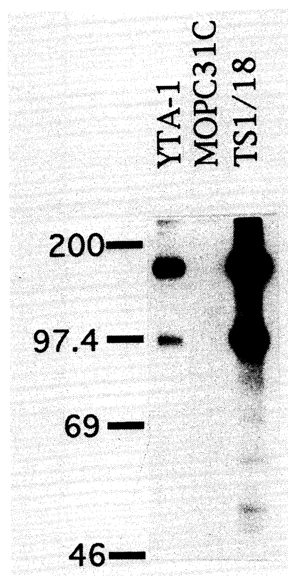


**Fig. 2.** Immunoprecipitation of YTA-1 antigen and LFA-1. $^{125}$I surface-labeled YT cells were incubated with YTA-1, MOPC31C IgG or TS1/18 mAb, cross-linked with DSP and then solubilized. The lysates were subjected to immunoprecipitation with rabbit anti-mouse IgG antibody and Pansorbin. All immunoprecipitates were analyzed by SDS–PAGE (8% gel) under reducing conditions.

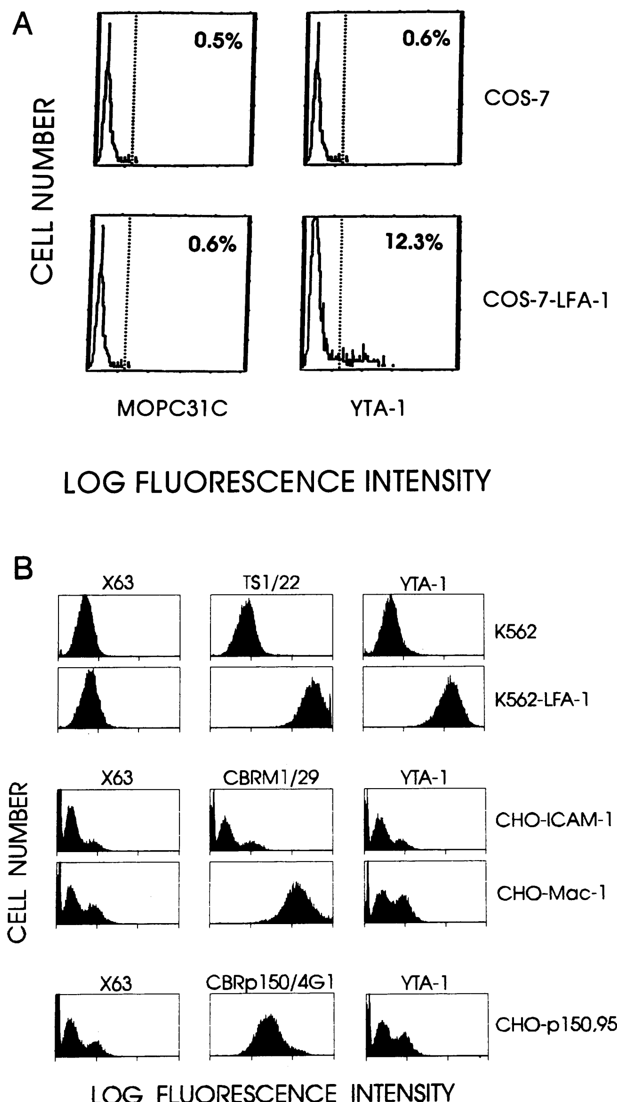


**Fig. 3.** (A) Expression of YTA-1 antigen on COS-7 cells transfected with LFA-1α and β cDNAs. COS-7 cells were transfected with both LFA-1α and LFA-1β cDNAs cloned in pCDM8. Two days after transfection, the transfected or untransfected COS-7 cells were stained with either MOPC31C IgG or YTA-1 mAb followed by the FITC-conjugated goat anti-mouse IgG antibody and analyzed by flow cytometry. The ordinate and abscissa represent the cell number and the fluorescence intensity on a logarithmic scale respectively. The values shown at the right-top represent the positive population. (B) Flow cytometric analysis of cells transfected with LFA-1, Mac-1 or p150, 95 cDNAs. Untransfected K562 cells, K562 cells that were transfected with both cDNAs for LFA-1α and LFA-1β, CHO cells that were transfected with both cDNAs for α and β chain of Mac-1 or p150, 95, and CHO transfectants of ICAM-1 cDNA were immunostained with X63 (non-binding control), TS1/22 (anti-LFA-1α), CBRM1/29 (anti-Mac-1α) or CBRp150/4G1 (anti-p150, 95α) and analyzed by flow cytometry. Representative histograms show the log fluorescence versus cell number.

we employed a cleavable cross-linker DSP to covalently couple YTA-1 to $^{125}$I-labeled cell surface proteins. Cells were then solubilized, and the immune complexes were precipitated by rabbit anti-mouse IgG antibody and Pansorbin. Under reducing conditions, the intramolecular disulfide bond of DSP should be cleaved so that the resulting bands of radioiodinated proteins reflect the molecular weight of antigen on SDS gels. YTA-1 antigen cross-linked to the mAb migrated as two bands of ~170 and 96 kDa, molecular weights of which correspond to those of LFA-1α and β chain respectively. As shown in Fig. 2, the peptides cross-linked to TS1/18 anti-LFA-1β were indistinguishable from those bound to YTA-1.

To confirm the relationship between YTA-1 antigen and LFA-1, we transfected COS-7 cells with LFA-1α and β expression vectors and determined the binding of YTA-1 to the transfectants by immunofluorescence. YTA-1 did not bind to untransfected COS-7 cells, but the cells transfected with both LFA-1α and β cDNAs were stained with YTA-1 (Fig. 3A). These data indicate that YTA-1 mAb recognizes LFA-1.

LFA-1 (CD11a/CD18), Mac-1 (CD11b/CD18) and p150, 95 (CD11c/CD18) constitute a family of integrins that share a common 95 kDa β subunit (CD18). To investigate which subunit of LFA-1 is recognized by YTA-1, flow cytometric analysis was performed using the cells transfected with CD11a/CD18, CD11b/CD18 or CD11c/CD18 cDNAs. K562 cells transfected with CD11a/CD18 cDNA were intensely stained by mAb TS1/22 (anti-CD11a) or YTA-1 (Fig. 3B). In CHO cells transfected with ICAM-1 cDNA, no significant staining was seen with either CBRM1/29 (anti-CD11b) or YTA-1 as compared with the negative control X63 staining. CHO cells transfected with CD11b/CD18 cDNA were stained strongly with CBRM1/29. In contrast, YTA-1 exhibited at most a minimal increase in staining of these cells compared with X63, far less than would be expected if YTA-1 recognized an

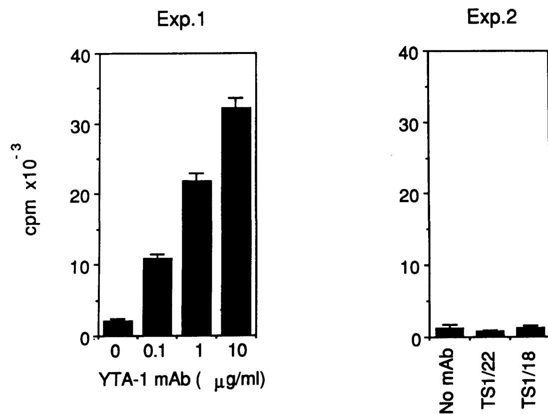


**Fig. 4.** Proliferative response of CD3⁻ LGL to YTA-1 mAb. Enriched CD3⁻ LGL were cultured in the presence of varying doses of YTA-1 mAb (Exp. 1) for 96 h and [$^3$H]thymidine incorporation was determined. In Exp. 2, LGL were cultured in the presence of 10 µg/ml of TS1/22 anti-LFA-1α or TS1/18 anti-LFA-1β mAb, or without mAb for 96 h. Similar results were obtained in three different experiments.

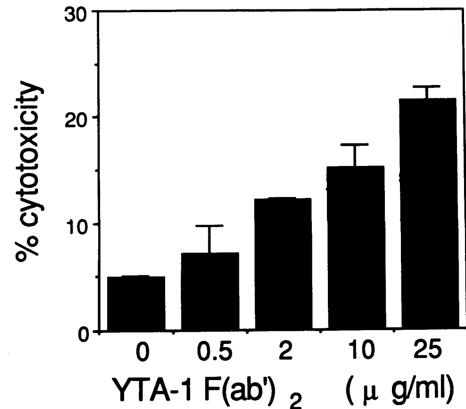


**Fig. 5.** Enhancement of NK cytotoxic acivity by YTA-1 mAb F(ab′)$_2$ fragments. PBMC (3×10$^6$) were cultured in 1 ml T medium containing the indicated concentration of YTA-1 F(ab′)$_2$ for 20 h, washed three times and tested for cytotoxicity against $^{51}$Cr-labeled K562 targets. Mean cytotoxicity of the triplicated data points at an E:T ratio of 5:1 are presented. The results were reproduced in three different experiments.

**Table 1.** Augmentation of NK cell activity by YTA-1 mAb independent of IL-2

| Treatment | Cytotoxicity (LU/10$^6$ cells) |
|---|---|
| None | 61 ± 12 |
| YTA-1 | 251 ± 18 |
| IL-2 | 197 ± 19 |
| YTA-1 + IL-2 | 275 ± 23 |
| Anti-IL-2 | 52 ± 7.8 |
| IL-2 + anti-IL-2 | 66 ± 9.2 |
| YTA-1 + anti-IL-2 | 237 ± 23 |

CD3⁻ LGL were treated with 10 µg/ml of YTA-1 mAb or 1000 U/ml of IL-2 in the presence or absence of anti-IL-2 mAb for 18 h prior to a cytotoxicity assay. Results are expressed as the mean LU ± SD. Similar results were obtained in three different experiments.

epitope formed solely by CD18. Similar results were obtained with CHO cells transfected with CD11c/CD18 cDNA. These data suggest that YTA-1 mAb recognizes LFA-1α (CD11a).

### *Activation of CD3⁻ LGL by YTA-1 mAb*

Since YTA-1 is mitogenic for unfractionated PBMC and brightly stains CD3⁻CD16⁺ NK cells (11), we determined whether this mAb is mitogenic for NK cells. Purified CD3⁻ LGL showed a dose-dependent proliferative response to YTA-1 (Fig. 4, Exp. 1). In the presence of the antibody, the number of cells increased until 30 days of culture confirming that YTA-1 mAb stimulated growth of NK cells (data not shown). In contrast, the other mAbs to LFA-1, TS1/22 and TS1/18, had no mitogenicity on NK cells (Fig. 4, Exp. 2). Immobilization on plastic dish enhanced the mitogenicity of YTA-1 by ~7-fold, but did not confer mitogenicity to TS1/22 or TS1/18 (data not shown). NK cells produce IFN-γ upon stimulation with cytokines or microbial infections (26). Thus we determined whether activation of NK cells with YTA-1 results in the production of IFN-γ. Culture of CD3⁻CD16⁺ NK cells in the presence of 20 µg/ml of YTA-1 for 48 or 96 h induced production of 2100–2400 pg/ml IFN-γ, while that with TS1/22 or no mAb resulted in undetectable levels (<15.6 pg/ml) of IFN-γ production. To address whether the proliferative response of NK cells is due to the production of cytokines induced by the antibody, we added neutralizing antibodies against IL-2, IFN-γ or IFN-α to the culture of LGL. The YTA-1-induced proliferation of LGL was not affected by saturating concentrations of any of the antibodies, suggesting that YTA-1 directly activates NK cells without involvement of these cytokines known to activate NK cells (data not shown).

It has been documented that monoclonal anti-LFA-1α and anti-LFA-1β, as well as anti-ICAM-1, i.e. antibody against a natural ligand for LFA-1, blocked cytotoxicity mediated by NK cells (7,8). Thus we tested the effects of YTA-1 mAb on NK cytotoxic activity. Addition of YTA-1 just prior to the cytotoxicity assay did not affect the NK activity (data not shown). However, purified CD3⁻CD16⁺ LGL showed enhanced cytotoxicity against K562 cells when the LGL were cultured with YTA-1 for 18 h prior to the assay (Table 1). The augmentation of cytotoxicity by 10 µg/ml of YTA-1 was comparable to that induced by 1000 U/ml of IL-2. LGL treated with YTA-1 were also cytotoxic to NK-resistant target cells including Daudi (data not shown). It was also found that a neutralizing antibody against IL-2 did not inhibit YTA-1-induced increase in cytotoxicity (Table 1). A neutralizing antibody against IFN-γ did not affect the augmentation of cytotoxicity by YTA-1 (data not shown). Considering the possibility that Fcγ receptors might mediate the effect of YTA-1, the F(ab′)$_2$ fragments of YTA-1 were tested for their ability to enhance NK cytotoxicity. Similar to the intact YTA-1 mAb, the F(ab′)$_2$ fragments enhancd the cytotoxicity against K562 target cells in a dose-dependent manner (Fig. 5). Thus, the augmentation of NK cell activity by YTA-1 does not appear to be due to reverse antibody-dependent cell-mediated cytotoxicity or activation through FcγRIII on NK cells, but to the stimulation of NK cells through LFA-1.

## Discussion

Data presented in this paper demonstrated that a mitogenic mAb YTA-1 binds to LFA-1. Since YTA-1 reacted with LFA-1 (CD11a/CD18) transfectants, but did not react with Mac-1 (CD11b/CD18) or p150, 95 (CD11c/CD18) transfectants, we speculate that YTA-1 recognizes LFA-1α (CD11a). However, it is possible that LFA-1β may contribute to the YTA-1 epitope. In fact, one of the two mAbs to LFA-1β, as well as one of the three mAbs to LFA-1α, almost completely blocked the YTA-1 staining. Our previous finding that YTA-1 down-regulated the high- and intermediate-affinity IL-2 receptors on YT, a human NK-like cell line, suggested that the antibody recognizes IL-2Rβ or molecules which were not IL-2Rβ itself but were associated with the multichain IL-2 receptor complex (11). The former possibility is unlikely since YTA-1 bound to MT-1 cells, which lack the expression of IL-2Rβ (12). In addition, the number of binding sites of [$^{125}$I]YTA-1 on YT cells was 10- to 20-fold more than that of IL-2Rβ as determined by Scatchard plot analysis (data not shown). We now believe that the 70–75 kDa band previously eluted from the YTA-1 column following incubation with YT cell lysate (11) was either a non-specific band or a proteolytic fragment of either LFA-1α or LFA-1β. The significance of the down-regulation of IL-2 receptors by YTA-1 remains to be determined by further investigation.

YTA-1 mAb was previously shown to be mitogenic for PBMC (11), but the lymphoid subpopulation responding to YTA-1 mAb was not defined. The present work demonstrated that the antibody induced proliferation of freshly isolated CD3$^-$ LGL. It was also found that 18 h incubation of CD3$^-$ LGL with the antibody enhanced the cytotoxicity of NK cells. Since LFA-1 is expressed on CD8$^+$ T cells, one may wonder that YTA-1 may activate CD8$^+$ cytotoxic T cells. Tumor-infiltrating lymphocytes, which consist mainly of CD8$^+$ T cells, also responded to YTA-1 with enhanced autologous tumor killing activity and proliferation (A. Uchida *et al.*, in preparation). Taken together, YTA-1 may be able to activate cytotoxic lymphocytes which strongly express LFA-1.

Consistent with previous reports (7,8), TS1/22 and TS1/18 inhibited NK cell activity when the mAb was added to the cytotoxicity assay system (data not shown). Under the same conditions, however, YTA-1 did not affect NK cytotoxicity. We speculate that the relatively low affinity of YTA-1 to LFA-1 might be relevant to the inability to block NK cytotoxicity. Interestingly, when NK cells were cultured for 18 h with TS1/22 or TS1/18, NK cytotoxicity remained unchanged (data not shown), in contrast to the cytotoxicity augmented by the preculture with YTA-1. Moreover, 96 h culture with YTA-1 resulted in the proliferation of CD3$^-$ LGL, while the other anti-LFA-1 did not induce proliferation. In addition, NK cells produced IFN-γ when cultured with YTA-1, but not with TS1/22. These findings suggest that YTA-1 may recognize a unique epitope of LFA-1 involved in the activation of NK cells. Accumulating evidence indicates that adhesion molecules including integrins transmit activation signals into the cells. LFA-1, in particular, is implicated in accessory signaling for the TCR since stimulation with anti-LFA-1 mAbs or ICAM-1 protein, a ligand of LFA-1, provides a co-stimulus for proliferative response (27–29) or programmed cell death (30) of T cells following CD3–TCR engagement. In addition, mAb reactive with a carbohydrate determinant of LFA-1 was shown to activate T cells (31). YT cells killed L cells transfected with both ICAM-1 and B7 cDNAs, suggesting that the collaboration of LFA-1 and CD28 can trigger MHC-unrestricted cytotoxicity (32). Nevertheless, no evidence has been presented which directly indicates that LFA-1 is involved not only in adhesion of NK cells with target cells but also in activation of NK cells. Notably, it was reported that an anti-LFA-1β mAb PNK-I inhibited cytotoxicity of NK cells without affecting the binding to target cells, suggesting that LFA-1 can mediate signal transduction in NK cells (33). The present work is the first, to our knowledge, to demonstrate that an anti-LFA-1 mAb induces proliferation and augments cytolytic activity of human fresh NK cells. It is important to address the physiologic significance of NK cell activation through LFA-1. We have observed that a protein tyrosine kinase(s) physically associated with LFA-1 in NK cells can be activated by the stimulation with the K562 NK-sensitive target cells but not with the P815 NK-resistant target cells (34). Thus, we hypothesize that LFA-1 may be involved in the signal transduction following target recognition. The activation of NK cells by YTA-1 might suggest the possibility that YTA-1 epitope may be involved in the target recognition by NK cells. However, LFA-1 may not act solely as the NK receptor that recognizes target cells for the following reasons. (i) LFA-1 is strongly expressed on CD8$^+$ cytotoxic T cell clones which can not mediate MHC-unrestricted cytotoxicity. (ii) Fresh NK cells did not mediate 'redirected cytotoxicity' against YTA-1 mAb-producing hybridoma (data not shown). As suggested by a previous report (32), it is likely that LFA-1 collaborates with an appropriate array of adhesion molecules such as CD2 to trigger NK cytotoxicity. Alternatively, LFA-1 may provide signals that render NK cells responsive to the stimulation through the putative NK receptors (1–6). This possibility might be consistent with the fact that preculture with YTA-1 for 18 h is required for augmentation of cytotoxicity. Biochemical characterization of molecules associated with LFA-1 in NK cells may be useful for elucidation of the mechanism of target recognition.

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## Abbreviations

| | |
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| DSP | dithiobis-succinimidyl-propionate |
| HBSS | Hanks' balanced salt solution |
| LFA-1 | leukocyte function-associated antigen |
| LGL | large granular lymphocytes |
| LU | lytic unit |
| NK | natural killer |
| PBMC | peripheral blood mononuclear cells |

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