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Induction of the 2B9 antigen/dipeptidyl peptidase IV/CD26 on human natural killer cells by IL-2, IL-12 or IL-15

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SUMMARY

Activation of human natural killer (NK) cells involves sequential events including cytokine production and induction of cell surface molecules, resulting in the enhancement of cytolytic activity. To delineate the activation process of NK cells, we generated murine monoclonal antibodies (mAbs) against YT, a human large granular lymphocyte/natural killer (LGL/NK) cell line. Among the mAbs reactive with YT cells, one mAb, termed 2B9, was noted because of the lack of reactivity with most of the human T- and B-cell lines tested. In fresh peripheral blood mononuclear cells (PBMC), however, the majority of cells expressing this antigen (Ag) were T cells but not $CD16^+$ nor $CD56^+$ NK cells. Since YT cells showed an activated phenotype expressing interleukin-2 (IL-2) receptor α chain, we examined whether 2B9 Ag could be induced on normal human peripheral blood NK cells by cytokines known to activate NK cells. The 2B9 Ag was induced on NK cells by IL-2, IL-12 or IL-15 while no induction was observed by interferon-γ (IFN-γ). Biochemical analysis showed that anti-2B9 mAb recognized a 115 kDa molecule in YT cells. A cDNA clone encoding the 2B9 Ag was isolated from a cDNA expression library of YT cells and its sequence was identical to CD26 cDNA although it was not of full length. Transient expression of the 2B9 cDNA on COS-7 cells revealed that this cDNA encodes the antigenic epitope(s) recognized by anti-2B9 mAb as well as Ta1, an anti-CD26 mAb. These results showed that the 2B9 Ag is identical to CD26, and demonstrated that CD26 is an activation antigen on $CD16^+$ $CD56^+$ NK cells inducible by IL-2, IL-12 or IL-15.

INTRODUCTION

Natural killer (NK) cells are a phenotypically and functionally distinct population of lymphocytes with characteristic morphology of large granular lymphocytes (LGLs). NK cells do not rearrange the T-cell receptor (TCR) genes or express the CD3–TCR complex but instead most NK cells express other triggering receptors including the low-affinity receptor for immunoglobulin G (IgG)/CD16. In addition, it is known that virtually all human NK cells express neural cell adhesion molecule (NCAM)/CD56 on their cell surface.[1,2] Functionally, NK cells have been defined as the major effector cells of non-adaptive natural immunity.[2,3] Recent evidence has indicated that NK cells recognize absence of self major histocompatibility complex (MHC) class I by their specified receptors. It is generally accepted, however, that NK cells can exert cytotoxicity against selected tumour and virus-infected cells without prior sensitization or as the effector cells of antibody (Ab)-dependent cellular cytotoxicity against Ab-sensitized target cells.[2,3]

NK cells exhibit enhanced cytolytic function on a variety of occasions including viral and other microbial infections.[4] Cytokines such as interferon-γ (IFN-γ) and interleukin-2 (IL-2) are thought to be involved in such activation processes.[5,6] It has been reported that IL-15 as well as IL-12 can also augment cytolytic activity of NK cells.[7,8] Activation of NK cells leads to production of IFN-γ and other cytokines, and phenotypical changes in their cell surface.[2] Namely, cell surface molecules such as MHC class II and IL-2 receptor α chain/Tac are absent or only weakly expressed in the resting state and are markedly induced on activation of NK cells.[9,10] It is a natural assumption that other unknown cell surface molecules are induced upon activation which may play important roles in activation process in NK cells.

We previously established a human LGL/NK cell line, YT,

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Abbreviations: Ag, antigen; CM, conditioned medium; DPP IV, dipeptidyl peptidase IV; FITC, fluorescein isothiocyanate; HIV, human immunodeficiency virus; IL-2R, interleukin-2 receptor; IFN-γ, interferon-γ; mAb, monoclonal antibody; PE, phycoerythrin; MHC, major histocompatibility complex; NK, natural killer; LGL, large granular lymphocyte; PBMC, peripheral blood mononuclear cells; TCR, T-cell receptor.

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expressing a large amount of intermediate affinity IL-2 receptors that are now recognized as heterodimers of IL-2 receptor β and γ chains.[11] IL-2 receptor α chain is also moderately expressed on YT cells and can be markedly up-regulated by cytokines such as IL-1 as well as various stimulants including the culture supernatants of ATL-2, human T-cell leukaemia virus-I (HTLV-I)-transformed T-cell line.[11] As YT cells represent an activation phenotype of human NK cells, we expected that additional activation Ags would be expressed on these cells. In order to delineate the activation process of NK cells, we have attempted to generate monoclonal antibodies (mAbs) recognizing novel NK activation Ags by immunizing mice with YT cells.

In the present study, we established a novel mAb, 2B9, recognizing a cell surface Ag expressed on YT cells but not on normal resting NK cells, and analysed the distribution and biochemical properties of the 2B9 Ag. Furthermore, molecular cloning of this Ag has been done to reveal unexpectedly that it is identical to CD26/dipeptidyl peptidase IV which is known to be a T-cell activation antigen. We here report that CD26 expression can be induced on peripheral blood NK cells by IL-2, IL-12 or Il-15.

MATERIALS AND METHODS

mAbs

Phycoerythrin (PE)-conjugated Leu-4a (anti-CD3), Leu-3a (anti-CD4), Leu-2a (anti-CD8), Leu-11c (Anti-CD16), Leu-16 (anti-CD20), Leu-19 (anti-CD56) and fluorescein isothiocyanate (FITC)-conjugated Leu-4a (anti-CD3), Leu-2a (anti-CD8), Leu-11c (anti-CD16), H107 (anti-CD23) were purchased from Becton Dickinson (San Jose, CA). The other mAbs used in the present study are as follows: anti-CD25/anti-IL-2 receptor α chain (provided by T. Uchiyama, Kyoto University, Japan), anti-CD26/anti-Ta1 (provided by E.L. Reinherz, Dana-Farber Cancer Inst., MA), MOPC21 mAb (mouse IgG1 with κ light chain, Sigma Immuno Chemicals, St. Louis, MO)

Cell culture

HUT102; ATL-2; MT1 (three HTLV-I-transformed T-cell lines), RPMI8866 (an Epstein–Barr virus (EBV)-transformed B lymphoid cell line), U937 (a monocytoid cell line), YT and 2B9 (hybridoma) were maintained in RPMI-1640 medium (Gibco-BRL, Grand Island, NY) supplemented with 10% fetal bovine serum at 37° with 5% CO_2. COS-7 cells were maintained in IMDM medium (Gibco-BRL) supplemented with 10% fetal bovine serum at 37° with 5% CO_2.

Establishment of anti-2B9 mAb

Hybridomas were generated by fusion of X63–1214 mouse myeloma cells with spleen cells from a BALB/c mouse immunized with YT cells, as described elsewhere.[12] mAbs reacting with YT cells were selected by flow cytometry. One of hybridomas, named 2B9, was noted because of its negative reactivity with other T- or B-cell lines examined. The isotype of mAb produced by 2B9 hybridomas was determined by using Immuno Select isotyping kit (Gibco-BRL). Its isotype was IgG1 with κ light chains. Anti-2B9 mAb was also labelled with FITC (Sigma,) or NHS–LC–biotin (Pieris, Foster City, CA) as previously described.[13]

Stimulation of YT cell lines

YT cells were cultured for 2 days in 5 ml of culture medium at 1×10^5 cells/ml with or without the culture supernatants of ATL-2 (provided by Dr Michiyuki Maeda, Kyoto University of Japan), an HTLV-I-transformed T cell line, which was grown in RPMI-1640 medium (Gibco-BRL) supplemented with 10% fetal bovine serum.[11] On day 1, the cultures of YT cells were diluted 1:2 with the same medium as initial to avoid over growth of YT cells. On day 2, the cells were washed once, stained with FITC-conjugated anti-2B9 mAb, anti-Tac/anti-IL-2 receptor a chain mAb, control MOPC21 mAb or FITC-conjugated MOPC21 mAb, and their surface fluorescence was analysed by flow cytometry. FITC-conjugated goat anti-mouse IgG mAb (Meloy Laboratories, Springfield, VA) was used as a secondary Ab to detect the binding of the FITC-unconjugated mAbs.

Flow cytometry

Direct and indirect immunofluorescence analyses were done using an Epics Elite flow cytometer (Coulter Co. Ltd, Hialeah, FL). To avoid cross-reactivity of secondary antibodies, mAbs conjugated with either FITC, PE or NHS–LC–biotin were used for multicolour immunofluorescence studies unless specified. NHS–LC–biotin conjugated mAbs were detected by the binding of the fluorescence substance streptavidin-RED 670 (Gibco BRL, Gaithersburg, MD) which absorbs the light at 488 nm and emits 670 nm red light and is detected as a third colour.

Stimulation of peripheral blood NK cells

Peripheral blood NK cell-enriched populations were prepared as follows. Peripheral blood mononuclear cells (PBMC) were isolated from heparinized venous blood from healthy individuals by Ficoll Paque (Pharmacia, Uppsala, Sweden) gradient centrifugation. Adherent monocytes were removed from PBMC by adherence to plastic tissue culture dishes for 1 hr at 37° and B cells were removed through a nylon wool column. Remaining cells were fractionated according to density on discontinuous gradient of Percoll as described.[14] After this procedure, $CD16^+$ NK cells were enriched to 70%. The remaining 20% cells were $CD3^+$ cells and 10% were $CD20^+$ cells. The recovered cells were cultured in a flat-bottom 96-well plate at a concentration of 10^6 cells/ml in 200 μl RPMI-1640 medium containing 10% fetal bovine serum with or without following concentration of cytokines: recombinant human IL-2 (Shionogi Pharmaceutical Co. Ltd, Osaka, Japan), 75 ng/ml (5 nM); recombinant human IL-12 (R & D Systems, Minneapolis MN), 150 ng/ml (1 nM); recombinant human IL-15 (provided by Immunex Research and Development Corp., Seattle WA), 10 ng/ml (0·6 nM); recombinant human IFN-γ (Chemicon International Inc., Temecula, CA), 1000 units/ml. The specific activities of all the cytokines used were checked and described by the providers. Then, cells were incubated for 2–4 days at 37° with 5% CO_2 and analysed by tri-colour flow cytometry using FITC-conjugated anti-CD16 mAb, PE-conjugated anti-CD56 mAb and NHS–LC–biotin conjugated anti-2B9 mAbs, as described above. The cells from three healthy individuals were examined.

Immunoprecipitation of the 2B9 Ag

YT cells (4×10^9) were externally labelled with ^{125}I by the lactoperoxidase-catalysed method using Enzymobeads

(BioRad, Richmond, CA), lysed with NP-40/deoxycholate lysis buffer (1% NP-40, 0·4% deoxycholate, 150 mM NaCl, 2 mM ethylene diamine tetra-acetic acid (EDTA), 1 mM PMSF, 1% aprotinin, 50 mM Tris–HCl, pH 7·4) or CHAPS lysis buffer (6 mM CHAPS, 150 mM NaCl, 2 mM EDTA, 1 mM PMSF, 1% aprotinin, 50 mM Tris–HCl, pH 7·4). Immunoprecipitation was done using anti-2B9 mAb or control MOPC21 IgG1 mAb as previously described[12] and the samples were analysed by sodium docecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) on a 8% polyacrylamide gel followed by autoradiography.

Affinity purification of the 2B9 Ag

YT cells (4×10^9) were lysed with 0·5% non-idet P-40 in 150 mM NaCl, 10 mM Tris–HCl, pH 7·2, containing a mixture of protease inhibitors at 4° and passed through a 22-gauge syringe several times to give rise to the starting lysate sample as described.[12] Generation of affinity columns and the purification procedures were described previously.[15] Briefly, 2·4 mg of mAbs were covalently coupled to 1 ml of Affi-Gel 10 affinity beads (BioRad Laboratories). The samples were applied to the columns with 200 μl bed volumes, washed, and recovered in 300 μl phosphate buffered saline. The 7 μl of recovered samples were run on a 7·5% polyacrylamide gel under reduced conditions, followed by silver staining to be visualized (Wako Pure Chemicals, Osaka, Japan).

cDNA cloning

A cDNA library of YT cells in pME18S expression vector was kindly provided by Dr Atsushi Miyajima (DNAX Research Institute, Palo Alto, CA).[16] cDNA cloning was done by transient expression in COS-7 cells and subsequent immunoselection with 2B9 mAb, as described elsewhere.[17] After four cycles of enrichment, individual plasmid clones were transfected to COS-7 cells by the DEAE–dextran method[17] and the expression of 2B9 Ag was examined on day 2 by flow cytometry. The insert DNA of the clone encoding 2B9 Ag was sequenced by dideoxy method using Sequenase version 1·0 sequencing kit (United States Biochemical, Cleveland, OH). CD23 cDNA in the expression vector, pKCR-εR2A,[18] was also transfected to COS-7 cells as a positive control for the transfection.

RESULTS

Establishment of 2B9 mAb and the augmentation of expression of the 2B9 Ag on YT cells

To study the activation Ags on NK cells and the cell surface structures involved in regulating NK functions, a BALB/c mouse was immunized with YT cells which represent an activated phenotype of NK cells. One of the hybridomas, named 2B9, was selected because of restricted reactivity of its supernatants with YT cells. Various cell lines were stained with anti-2B9 mAb or isotype-matched control MOPC21 mAb and subjected to flow cytometry. Anti-2B9 mAb reacted with YT cells but not with other human cell lines tested including a monocytoid cell line (U937), a B-cell line (RPMI8866) and HTLV-I (+) T-cell lines (HUT102, ATL-2, MT1) (data not shown).

Based on the hypothesis that NK activation Ags are expressed on YT cells and their expression is further up-regulated upon stimulation, we investigated whether the 2B9 Ag can be up-regulated as well, using the culture supernatants of ATL-2 that has been shown to up-regulate IL-2 receptor α chain on YT cells. In the experiments shown in Fig. 1, the up-regulation of 2B9 Ag was compared with that of IL-2 receptor α chain. Although the levels of augmentation of the 2B9 Ag was less than that of IL-2 receptor α chain, the expression of 2B9 Ag on YT cells was also augmented by the incubation with the conditioned medium.

Induction of the 2B9 Ag on CD16$^+$ CD56$^+$ NK cells

We next examined the expression of 2B9 Ag in normal human PBMC. The majority of the cells expressing 2B9 Ag were CD3$^+$ T cells (Fig. 2). The dual-colour immunofluorescence analysis revealed that the 2B9 Ag was expressed on a subpopulation of CD4$^+$ T cells as well as CD8$^+$ T cells. A subpopulation of CD20$^+$ B cells also expressed the 2B9 Ag.

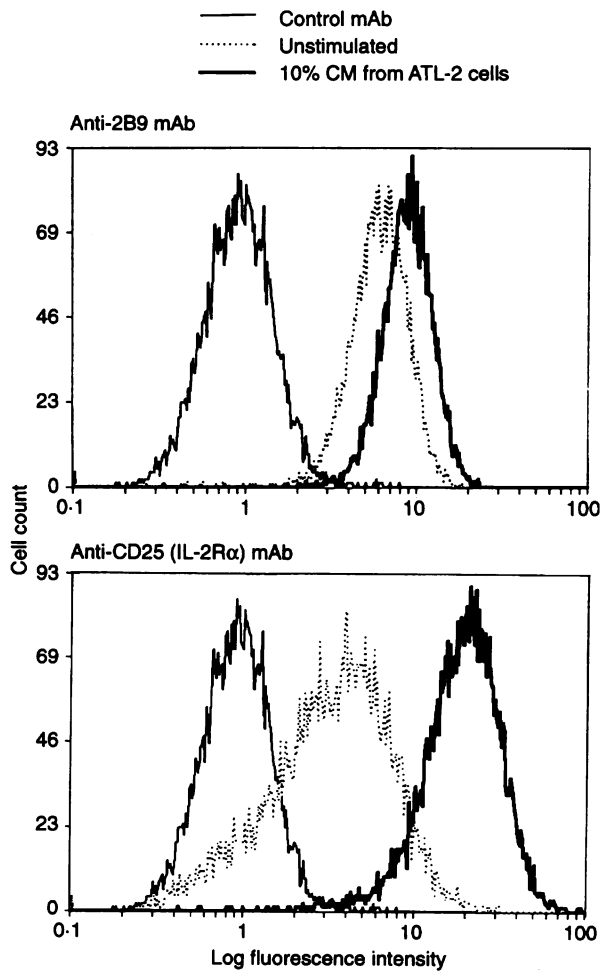

Figure 1. Up-regulation of the 2B9 Ag on YT cells. YT cells were cultured for 2 days in the presence or absence of 10% conditioned medium (CM) of ATL-2. The cell surface 2B9 Ag was analysed by flow cytometry. CD25 (IL-2 receptor α chain/Tac) was also monitored as a control for the stimulation. The isotype-matched MOPC21 mAb was used as a control mAb. Data are representative of four separate experiments.

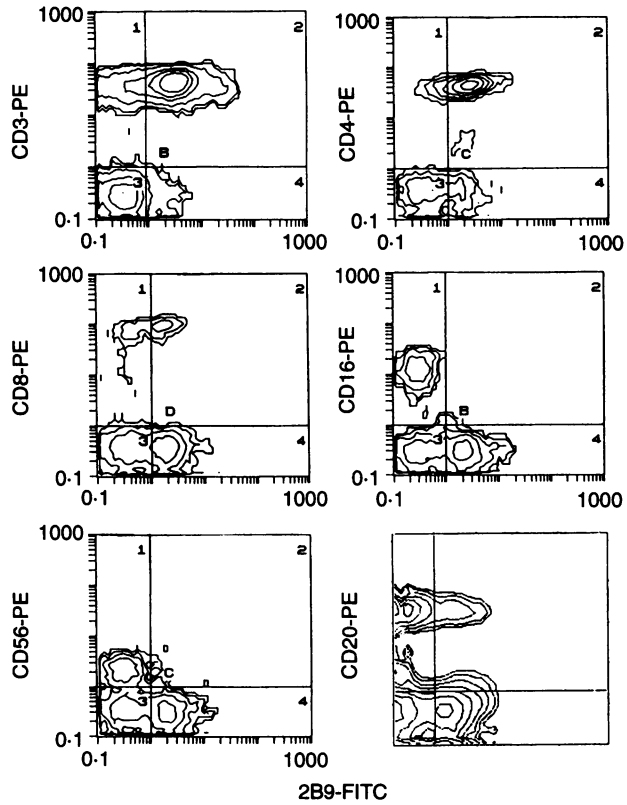

Figure 2. Reactivity of anti-2B9 mAb with unfractionated PBMC. PBMC were stained with the indicated mAbs and subjected to dual-colour immunofluorescence analysis.

Despite the expression of the 2B9 Ag on YT cells, it was not significantly detected on $CD16^+$ or $CD56^+$ NK cells.

Since YT has an activated phenotype of NK cells, it seemed possible that the 2B9 Ag would be induced on peripheral blood NK cells upon proper activation. Thus, we examined the effect of cytokines that are known to activate NK cells. NK cell-enriched populations from three healthy individuals were cultured with IL-2, IL-12, IL-15 or IFN-γ and the expression of 2B9 Ag was analysed by tri-colour flow cytometry. A representative experiment is shown in Fig. 3. On days 2–4, the 2B9 Ag was significantly induced on $CD16^+$ $CD56^+$ NK cells by IL-2, IL-12 or Il-15. No significant induction of the 2B9 Ag was observed even at very high doses (1000 units/ml) of IFN-γ. These data demonstrate that the 2B9 Ag is an activation antigen on NK cells that is induced by IL-2, IL-12 or Il-15.

Characterization of the 2B9 Ag

Biochemical characterization of the 2B9 Ag was carried out by immunoprecipitation as well as affinity purification. YT cells were externally labelled with 125I and subjected to immunoprecipitation using 2B9 mAb. SDS–PAGE analysis of the immunoprecipitates showed that the apparent MW of 2B9 Ag is $\approx$115 kDa (Fig. 4a), which was consistent with the results of affinity purification of 2B9 Ag from the YT cell lysates. Silver staining of the eluates from the anti-2B9 affinity column showed a specific band of 115 kDa. The bands of about 66 kDa seemed to be non-specific because it was also detected with MOPC21 control columns (Fig. 4b). These results indicate that 2B9 Ag is a single component of about 115 kDa.

Cloning and characterization of a cDNA encoding the 2B9 Ag

A cDNA encoding the 2B9 Ag was isolated by using the expression cloning technique as described in materials and methods. A plasmid clone with a 1·2 kb cDNA insert was isolated that encodes the antigenic epitope recognized by 2B9 mAb. The nucleotide sequence of the insert was analysed from the both ends and subjected to homology search. The result indicated that 2B9 cDNA is identical to CD26 cDNA. Restriction enzyme mapping also revealed that the restriction pattern of 2B9 cDNA is identical to that of the 5′ half of entire CD26 cDNA deduced from its nucleotide sequence (data not shown). The full length of CD26 cDNA has been reported to be 2·9 kb with about 2·3 kb open reading frame encoding a 110–115 kDa glycoprotein.[19] Transient expression of the isolated clone in COS-7 cells indicated that this cDNA clone also encodes the antigenic epitope recognized by Ta1, the first described anti-CD26 mAb (Fig. 5). Flow cytometric analysis of unfractionated PBMC shown in Fig. 2 indicated that the staining pattern with 2B9 mAb was essentially the same as that with Ta1 mAb.[20,21] These results clearly indicate that the 2B9 Ag is identical to CD26 and that both the 2B9 and Ta1 epitopes are encoded in the 5′ side 1·2 kb part of CD26 cDNA.

DISCUSSION

In the present study, we established 2B9 mAb which recognizes an activation Ag on NK cells. Characterization of 2B9 Ag has shown that it is identical to CD26/dipeptidyl peptidase IV (DPP IV; EC 3.4.14.5) which is an ectopeptidase specifically cleaving N-terminal dipeptides with either L-proline or L-alanine at the penultimate position.[22,23] A number of immunologically relevant substrates, such as IL-1β, IL-2, IL-6, lymphotoxin, transferrin, complement factor B, and other, possess the N-terminal X-Pro moiety and are potential substrates for DPP IV.[22,23] CD26/DPP IV is known as a T-cell activation antigen and also expressed on epithelial cells of various tissues including the liver, kidney and intestine.[20,24,25] It has been suggested that CD26 plays a key role in activation on a subset of $CD4^+$ T helper cells.[21,26,27] In these T cells, CD26 is known to be associated with CD45, a tyrosine phosphatase, and adenosine deaminase, both of which have been suggested to be involved in signal transduction.[28,29]

CD26 is not expressed on most of the peripheral blood NK cells except for a very minor subset of NK cells that is CD3–CD16–CD56 $bright^+$ subset, representing less than 1% of PBMC.[30] By contrast, NK cells activated with IL-2 or NK cell clones maintained *in vitro* have been reported to express CD26.[31,32] Despite these observations, the regulation of CD26 expression in the majority of peripheral blood NK cells remains unclear. In the present study, we demonstrated that CD26 is inducible on $CD16^+$ $CD56^+$ peripheral blood NK cells by IL-2, IL-12 or Il-15. We observed reproducibly that IL-2 or IL-15 induced CD26 expression more efficiently than IL-12 around day 4. It is possible that IL-2 and IL-15 which are both more potent growth factors than IL-12 for NK cells may

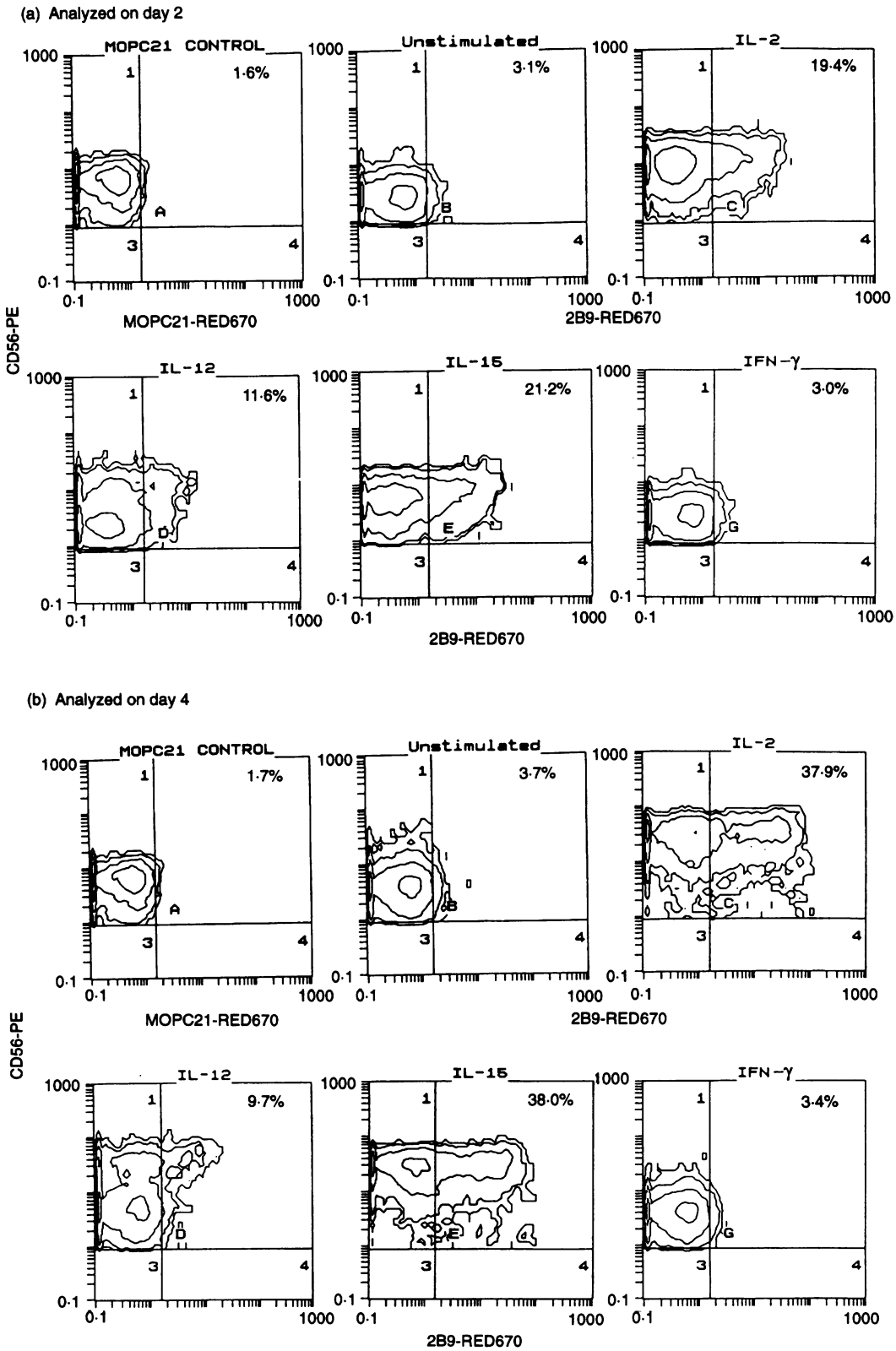

Figure 3. Induction of the 2B9 Ag on CD16$^+$ CD56$^+$ NK cells. The NK cells from three healthy individuals were cultured with or without following concentrations of cytokines as described in materials and methods: IL-2, 5 nM; IL-12, 1 nM; IL-15, 10 ng/ml; IFN-γ, 1000 units/ml. The cells were analysed by tri-colour flow cytometry. CD16$^+$ CD56$^+$ cells were gated and the expression of the 2B9 Ag was shown in relation to CD56. MOPC21 mAb was used as a control mAb. The results shown are representative of three independent experiments. (a) analysed on day 2. (b) analysed on day 4.

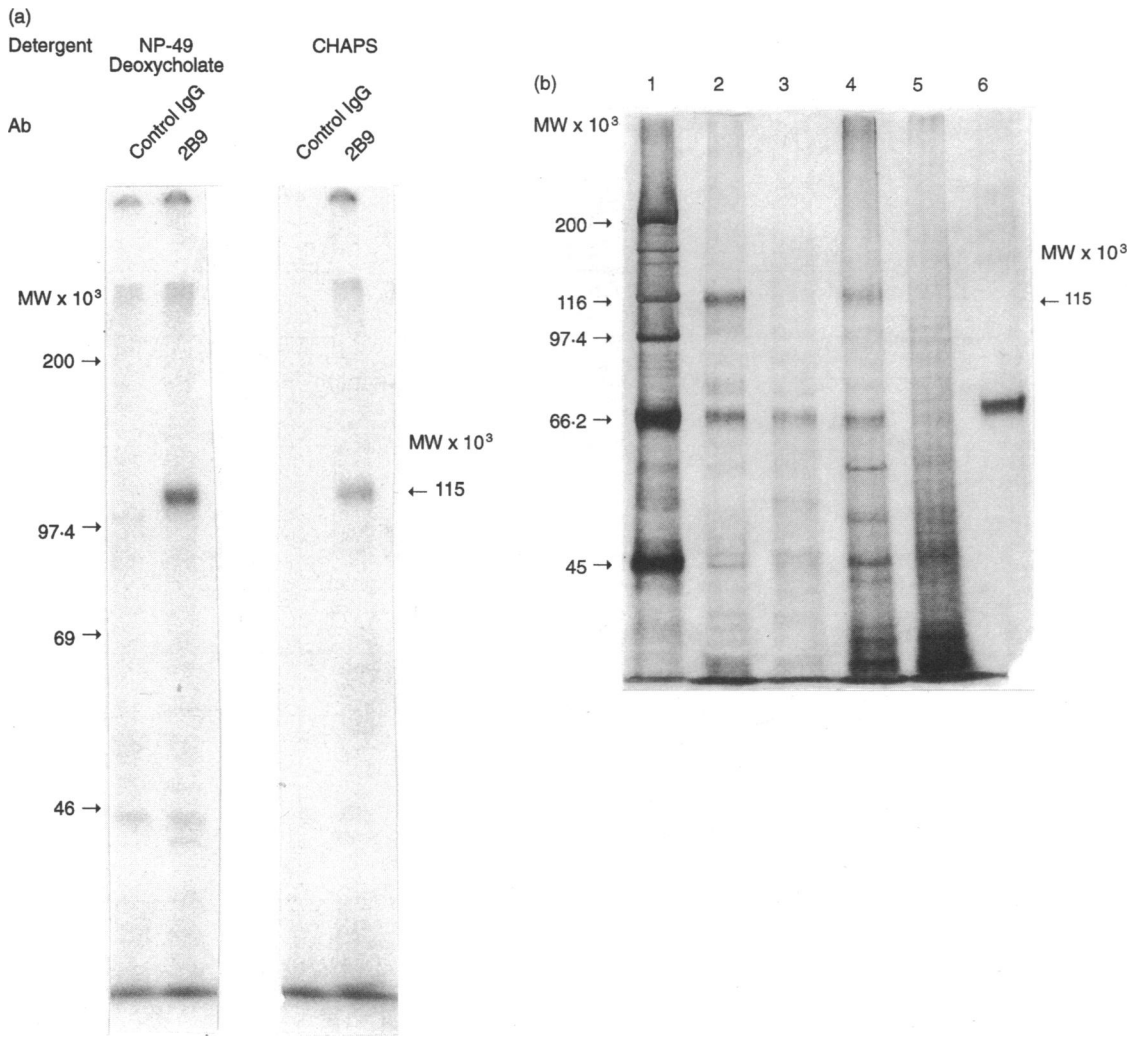

Figure 4. (a) Immunoprecipitation of the 2B9 Ag from YT cells. YT cells were externally labelled with 125I, lysed with the indicated detergent, immunoprecipitated using 2B9 mAb or control MOPC21 IgG1 mAb. The immunoprecipitates were analysed by 8% SDS–PAGE gel followed by autoradiography. (b) Affinity column purification of the 2B9 Ag from YT cells. YT cells were lysed with detergent and the solubilized fraction was applied to the affinity column of 2B9 mAb or control MOPC21 IgG1 mAb. After washing, the samples were eluted and run on 7·5% SDS–PAGE. Results of two representative purifications are shown. Lane 1, high molecular weight markers; lanes 2 and 4, anti-2B9 mAb column purified YT lysate; lanes 3 and 5, control MOPC21 mAb column purified YT lysate; lane 6, bovine serum albumin MW marker.

have expanded $CD26^+$ populations. Therefore, the difference may be partly due to the change of populations rather than real induction. It is also notable that the 2B9 Ag/CD26 is constitutively expressed on YT, a human LGL/NK cell line having an activated phenotype of NK cells. Although CD26 is known as a T-cell activation antigen, about half of the resting peripheral blood T cells already express CD26. Thus, these data indicate that CD26 is also an activation antigen for NK cells, and its expression is more tightly regulated in NK cells than in T cells. Since CD26 is known to have a binding affinity for collagen and thought to be involved in cellular adhesion to extracellular matrix proteins,[33,34] the induction of CD26 Ag on activated NK cells may be related to efficient recruitment and tissue infiltration, resulting in enhancement of target cell killing in the lesion.

As to the intracellular signals that induce CD26 expression, IL-2 and IL-15 are likely to use common or similar pathways because the receptors for IL-2 and IL-15 share the β chain and the common γ chain involving Jak1/Jak3 and (Stat3)/Stat5.[7,35] On the other hand, IL-12 receptor system is believed to have signal transduction pathways involving Jak2/Tyk2 and Stat3/Stat4.[35] It is interesting that IFN-γ did not induce the CD26, although IFN-γ has potent activating effects on NK cells. This might be due to the distinct activation mechanism of the IFN-γ receptor known to activate Jak1/Jak2 and Stat1.[35] However, further studies are required to elucidate the molecular mechanism of regulation of CD26 expression and determine whether or not the induction of CD26 can be explained simply by activation of a certain combination of Jak(s) and Stat(s).

Despite the fact that the cDNA we cloned was not full length, it provides some insights into the localization of the antigenic epitopes. We showed that 5′ side 1·2 kb part of CD26 cDNA is sufficient to form the Ta1 epitope and the 2B9

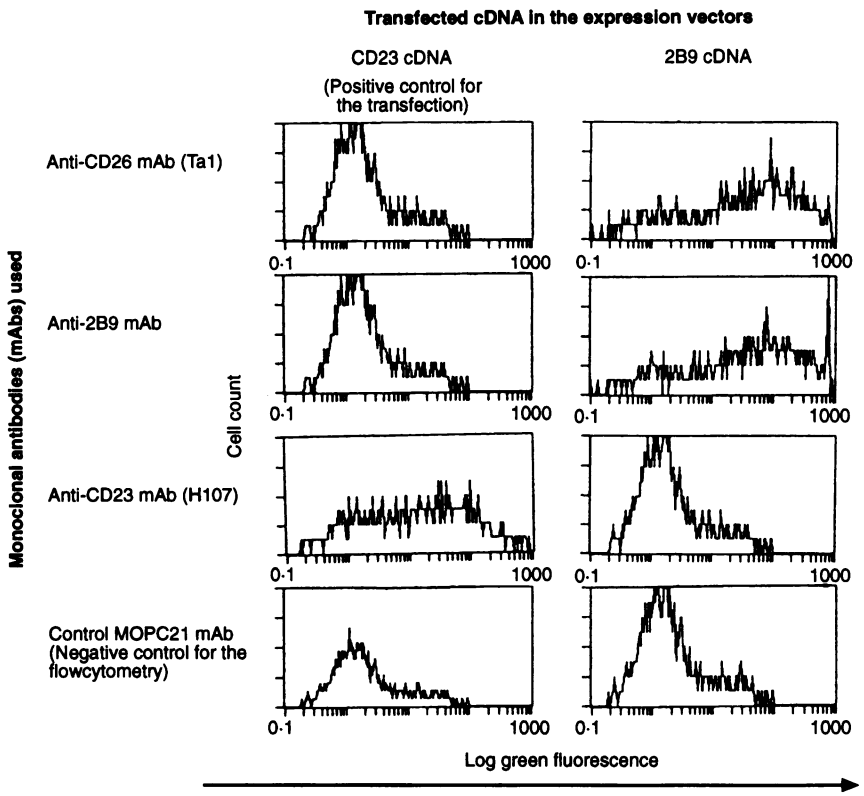

Figure 5. cDNA cloning and expression of the 2B9 Ag. The cloned cDNA encoding the 2B9 Ag in pME18S vector or control CD23 cDNA were transiently expressed on COS-7 cells by DEAE–dextran transfection. The transfectants were subjected to flowcytometric analysis with the following mAbs: anti-CD23 (H107); anti-2B9; anti-CD26 (Ta1); MOPC21 control mAb.

epitope on the cell surface. It has been published that the 2·9 kb full-length CD26 cDNA with 2·3 kb open reading frame encodes a type II transmembrane protein consisting of 766 amino acids residues.[19] Comparing the sequence of the isolated 1·2 kb cDNA with that of the full length cDNA, it is presumed that the cDNA we isolated encodes an incomplete protein which lacks the outer half of the extracellular domain of CD26. Thus, the Ta1 and the 2B9 epitopes may be located relatively close to each other, although we need further analysis to pinpoint the antigenic epitopes. In accordance with this assumption, 2B9 mAb and Ta1 mAb interfere with each other competitively in flow cytometric analysis (data not shown).

Human immunodeficiency virus (HIV)-Tat protein is known to be essential for transactivation of HIV viral genes as well as viral replication and can also be detected in the sera of HIV patients.[36–38] Recent studies have shown that soluble HIV-Tat protein directly binds to CD26 expressed on T cells, and suppresses Ag-specific T-cell response by inhibiting TCR/CD3 mediated signal transduction.[37–39] HIV-Tat protein has been reported to strongly inhibit lymphocyte proliferation induced by tetanus toxoid or *Candida* antigens by 66–97% at nanomolar concentration of HIV-Tat. It has been reported that the Ag-specific suppression and the binding of HIV-Tat to CD26 were blocked by the treatment with anti-Ta1 mAb and not by 1F7, another anti-CD26 mAb *in vitro*.[37] Thus, mAbs have been used to explore the functional domains of CD26 protein, and accordingly, 2B9 mAb may also serve as a useful tool to dissect the mechanism of Tat-mediated immunosuppressive effects on T cells. It has been reported that NK function is often suppressed in HIV-infected individuals.[40] Our finding of the CD26 expression on activated NK cells may lead to the idea that one of the mechanisms of impaired NK function in HIV infection may be due to the affection of activated NK cells by HIV-tat protein.

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