A monoclonal antibody, mNI-58A, against CD11a induces homotypic cell aggregation and promotes interferon-γ production by the human NK-like cell line, KHYG-1

Nobunao IKEWAKI Hidekazu TAMAUCHI *

Abstract

A monoclonal antibody (mAb), designated as mNI-58A, against CD11a (leukocyte function-associated antigen-1 α; LFA-1 α) was established in our laboratories by immunizing mice with the lipopolysaccharide (LPS)-stimulated human monocyte-like cell line, U937. This mAb specifically induced homotypic cell aggregation of the human NK-like cell line, KHYG-1. This mNI58A-induced homotypic cell aggregation was markedly blocked by the addition of an optimal concentration of a conventional protein kinase C (cPKC) inhibitor, Go6976, and was completely blocked by the addition of an optimal concentration of a novel protein kinase C (nPKC; a PKC delta isoenzyme) inhibitor, Rottlerin. Interestingly, KHYG-1 cells effectively promoted interferon-γ (IFN-γ) production in the culture supernatants of the homotypic cell aggregations of KHYG-1 cells induced by mNI-58A. These findings strongly indicate that CD11a mAb (mNI-58A) has some unique properties and may be useful for analyzing the cell-to-cell interactions of NK cells in several human immune responses.

Key words : CD11a, interferon-γ (IFN-γ), KHYG-1 cells, mNI-58A

Introduction

Adhesion molecules are not only involved in cell adhesion/aggregation, they also participate in the signaling of cellular information in intercellular communication networks and thereby control homeostasis throughout the entire body [1]. Hence, adhesion molecules play an important role in the physiological actions and the broad regulation of the immune system [2].

CD11a, leukocyte function-associated antigen-1 α (LFA-1 α) molecule, is a member of the integrin family of adhesion proteins and is mainly expressed on lymphocytes (T and B cells), monocytes, macrophages and granulocytes [3,4]. CD11a was initially identified by monoclonal antibodies (mAbs) that inhibit cell lysis induced by cytotoxic T cells or NK cells by preventing cell-to-cell interactions [5]. Since then, it has become clear that CD11a acts as a cellular adhesion molecule mediating a variety of heterotypic and homotypic cell aggregations.

Certain CD11a mAbs have been reported to induce cell proliferation, differentiation, and cell adhesion/aggregation in human and mouse lymphoid cells, suggesting a key role of CD11a in cell-to-cell interactions [6]. In addition, CD11a has also been implicated in signal transduction to cells upon binding of either specific mAbs or counter receptor intercellular adhesion molecules (ICAMs), such as ICAM-1, ICAM-2 and ICAM-3 [7,8]. Moreover, several reports have shown that the CD11a molecule on the human T cell line is closely associated with cytoskeletal rearrangement [9,10]. These findings suggest that CD11a may be involved in the signaling transduction of cytoskeletal rearrangement in several lymphocytes, monocytes and granulocytes [2]. However, the functional actions of CD11a on NK cells remain poorly understood.

We previously established a novel CD11a mAb, designated as mNI-58A, and reported some properties of its serological reactivity [11]. In the present study, we obtained evidence that mNI-58A induces homotypic cell

Department of Animal Pharmaceutical Science, Kyushu University of Health and Welfare School of Pharmaceutical Sciences, and Institute of Immunology, Takahashi Educational Institute, 1714-1 Yoshino-cho, Nobeoka, Miyazaki 882-8508, Japan
*Department of Microbiology, Kitasato University School of Medicine, 1-15-1 Kitasato, Sagamihara, Kanagawa 228-8555, Japan

aggregation and promotes interferon-γ (IFN-γ) production by the human NK-like cell line, KHYG-1, suggesting that the CD11a recognized by mNI-58A has some unique properties associated with the regulation of NK cells in some human immune responses.

Materials and Methods

Reagents

A conventional protein kinase C (PKC) inhibitor, Go6976, and a novel PKC (a PKC delta isoenzyme) inhibitor, Rottlerin, were purchased from Sigma Chemical, St Louis, MO. An enzyme-linked immunoassay (EIA) kit for interferon-γ (IFN-γ) was purchased from MBL Co. (Nagoya Japan). Recombinant interleukin-2 (rIL-2) was purchased from Cosmo Bio Co. (Tokyo Japan).

Cell line

The human NK-like cell line (KHYG-1)[12] used in the present study was supplied by the Japanese Research Resource Bank (Tokyo, Japan). KHYG-1 cells were cultured in RPMI 1640 medium (GIBCO) supplemented with 10mM HEPES buffer, 2mM glutamine, 10% fetal calf serum (FCS) and 50ng/mL of rIL-2 (referred to as complete medium).

Antibodies

The monoclonal antibodies (mAbs) mNI-58A (mouse IgG1; anti-CD11a)[11] and mNI-11 (mouse IgG1; anti-CD93)[13] were established in our laboratories. Normal mouse IgG1 antibody and fluorescence isothiocyanate (FITC)-conjugated goat anti-mouse IgG antibody were purchased from MBL Co. (Nagoya, Japan).

Detection of cell surface antigens

The detection of antigens on KHYG-1 cells was achieved using the indirect immunofluorescence method. Incubation with 20% normal human serum (NHS) for 30 min at 4℃ was performed first to block the Fc receptor for IgG, followed by incubating the cells with mAbs at an optimal concentration for 40 min at 4℃. After washing twice with phosphate-buffered saline (PBS), the cells were incubated with an FITC-conjugated goat anti-mouse IgG antibody for 20 min at 4℃. They were washed twice and were resuspended in PBS. Negative controls (NCs) were provided by incubating with isotype-matched normal mouse IgG1 antibody. The percentage of cells that stained positive for the mAbs was analyzed using a fluorescence-activated cell sorter (FACScan, Becton Dickinson).

Assay for homotypic cell aggregation

The cells were washed three times with complete medium and resuspended at a concentration of $5x10^5$/mL; 0.2mL of the cell suspension was then distributed into the wells of a 96-well microplate. Isotype-matched normal mouse IgG1 (5μg/mL); the CD11a mAb, mNI-58A (5μg/mL); or the CD93 mAb, mNI-11(5μg/mL) were added with or without the cPKC inhibitor, Go6976, and the nPKC inhibitor (a PKC delta isoenzyme), Rottlerin, and the plates were incubated at 37℃ for 18 hrs. The cells were then examined for aggregation under a phase-contrast microscope. Homotypic cell aggregation was scored in a semi-quantitative manner using the method described by Rothlein and Springer[14]. The scores ranged from 0 to 5, with 0 indicating essentially no cell clusters; 1+, less than 10% of the cells in clusters; 2+, 11-49% of the cell clusters in aggregates; 3+, 50-100% of the cell clusters in aggregates; 4+, nearly 100% of the cells in large clusters of aggregates; and 5+, all of the cells in very compact aggregates. The average of triplicate wells was used as the final score. Photographs were taken using an Olympus camera body under a phase-contrast microscope. The experiments were repeated at least five times.

Assay for IFN-γ in the culture supernatants

The detection of IFN-γ in the culture supernatants from the KHYG-1 cells cultured with or without mNI-58A (5μg/mL) for 18 hrs was performed using an EIA kit. The experiments were repeated three times.

Statistical analysis

The statistical analysis was performed using the Student *t*-test. Differences were considered significant when the *P* value was less than 0.05.

Results and Discussion

We have established a novel mouse mAb (mNI-58A) against CD11a (LFA-1α) with a molecular weight of about 180 kDa; some serological reactive patterns of mNI-58A were analyzed in our previous report [11]. In the present study, we investigated several biological functions of mNI-58A, such as cell morphological changes and IFN-γ production, in the human NK-like cell line, KHYG-1.

First of all, we examined the expression patterns of CD11a and CD93 on KHYG-1 cells using a CD11a mAb (mNI-58A), a CD93 mAb (mNI-11), and flow cytometry. As shown in Figure 1, both CD11a (90.7% positive; panel A) and CD93 (98.3% positive; panel B) were strongly expressed on KHYG-1 cells. CD11a is a member of the β2 integrin family of adhesion molecules and is mainly expressed on lymphocytes (T and B cells), monocytes, macrophages, granulocytes and NK cells [1, 4, 6]. It plays an important role as a receptor for the regulation of various immune responses, such as cell activation/proliferation and immunoregulatory cytokines production [15]. On the other hand, CD93, a receptor for complement component 1 (a subcomponent q phagocytosis [C1qRp]), is selectively expressed by myeloid cell lineages such as monocytes, granulocytes [16, 17] and is involved in the C1q-mediated enhancement of phagocytosis against various foreign antigens and apoptotic cells in innate immune responses [18].

Next, we investigated the effects of mNI-58A on the induction of homotypic cell aggregation by KHYG-1 cells. KHYG-1 cells were cultured for 18 hrs in complete medium containing isotype-matched normal mouse IgG1 (5 μg/mL), mNI-58A (5 μg/mL), or mNI-11 (5 μg/mL). As shown in Figure 2, mNI-58A (panel C) dramatically induced homotypic cell aggregation by the KHYG-1 cells (score, 5+). Isotype-matched normal mouse IgG1 (panel A) or mNI-11 (panel B), however, did not induce cell aggregation by KHYG-1 cells (score, 0 for both). In addition, the F(ab') and $F(ab')_2$ fragments of mNI-58A (5 μg/mL) also induced homotypic cell aggregation by KHYG-1 cells under the same conditions (data not shown). These findings indicate that the KHYG-1 cell aggregation induced by mNI-58A did not depend on binding to the Fc receptor for IgG or an antigen defined by mNI-58A cross-linking.

Some homotypic cell aggregations have been reported to depend on intracellular signaling pathways mediated by protein kinases, such as protein kinase C (PKC), protein kinase A (PKA) or protein tyrosine kinase (PTK) [19, 20]. Therefore, we also investigated the effect of protein kinase on KHYG-1 cell aggregation in addition to using several inhibitors. As shown in Figure 3, the aggregation induced by mNI-58A (panel B; score, 5+) was markedly blocked in the presence of a cPKC inhibitor, Go6976 (1.5 μM; panel C; score, 2+), and was completely blocked in the presence of an nPKC (delta isoenzyme) inhibitor, Rottlerin (1.5 μM; panel D; score, 0). Interestingly, Rottlerin blocked this aggregation more strongly than Go6976.

PKC molecules are known to regulate both positive and negative signal transduction pathways for the initiation and homeostasis of immune response systems. They represent a large family of structurally related serine/threonine protein kinases that are categorized into three major subgroups: the cPKC isoenzymes (cPKCs) that are Ca^{2+} and diacylglycerol (DAG) dependent, the nPKC isoenzymes (nPKCs) that are Ca^{2+} independent but DAG responsive, and the atypical PKC isoenzymes (aPKCs) that require neither Ca^{2+} nor DAG for activation. In addition, the cPKCs and nPKCs, but not the aPKCs, are known to rapidly respond to phorbol myristate acetate (PMA) treatment [19, 20].

Rottlerin, an nPKC inhibitor used in this study, is a unique drug that has been widely used as a pharmaceutical agent to analyze intracellular signal transduction based on its phosphorylation by protein kinases in various biological and immunological functions [21, 22]. Rottlerin exerts an inhibitory effect against the PKC delta isoenzyme and can regulate several biological phenomena such as the cell surface expression of antigens, cytokine production, cell adhesion/aggregation, and cell apoptosis [23]. Our studies revealed that the nPKC inhibitor Rottlerin blocked the homotypic cell aggregation of KHYG-1 cells more strongly than the cPKC inhibitor Go6976, suggesting that nPKC is mainly and strongly mediated in the intracellular signal transduction of KHYG-1 cells generated by mNI-58A.

We also investigated IFN-γ production from KHYG-1 cells in the presence of mNI-58A and homotypic cell aggregation. KHYG-1 cells spontaneously produce IFN-γ into the culture supernatant, as previously described [12]. The detection of IFN-γ in the culture supernatants of the KHYG-1 cells cultured with or without mNI-58A (5 μg/mL) was performed using an EIA kit. Figure 4 shows that IFN-γ production was promoted in KHYG-1 cells

cultured with (3.31±0.43 U/mL, on average) or without (1.55±0.21 U/mL, on average) mNI-58A. The difference between the supernatant from KHYG-1 cells cultured in the presence and that from cells cultured in the absence of mNI-58A was significant (P<0.01). On the other hand, although the data is not shown, PMA strongly enhanced IFN-γ production (22.9±1.52 U/mL, on average) from KHYG-1 cells into the culture supernatants. Therefore, KHYG-1 cells effectively augmented IFN-γ production into the culture supernatants, underlying the homotypic cell aggregation induced by mNI-58A. This finding suggests that mNI-58A effectively activated IFN-γ production in KHYG-1 cells via mainly an nPKC pathway, since the promotion of IFN-γ production from KHYG-1 cells induced by mNI-58A was significantly blocked by the addition of the nPKC inhibitor Rottlerin (data not shown).

In conclusion, we have provided evidence that a novel mNI-58A mAb against CD11a (LFA-1 α) induces homotypic cell aggregation by the human NK-like cell line KHYG-1, ultimately leading to the promotion of IFN-γ production. These findings strongly indicate that the immunological action of mNI-58A is critical and that this mAb may be useful for analyzing the biological functions of NK cells in various human immune responses. Further analyses are needed to demonstrate the detailed mechanism(s) of this finding at the cellular and molecular levels.

References

1 Larson, R.S, Springer, T.A.: Structure and function of leukocyte integrins. Immunol. Review 114: 181-217, 1990.

2 Isobe, M., Yagita, H., Okumura, K., et al.: Specific acceptance of cardiac allograft after treatment with antibodies to ICAM-1 and LFA-1. Science 255: 1125-1127, 1992.

3 Dustin, M.L., Springer, T.A.: T-cell receptor cross-linking transiently stimulates adhesiveness through LFA-1. Nature 341: 619-624, 1989.

4 Yamada, A., Kaneyuki, T., Torimoto, Y., et al.: Signaling from LFA-1 contributes signal transduction through CD2 alternative pathway in T cell activation. Cell. Immunol. 142: 145-158, 1992.

5 Krensky, A.M., Sanchez, M.F., Robbins, E., et al.: The function significance, distribution and structure of LFA-1, LFA-2, and LFA-3: Cell surface antigens associated with CTL-target interactions. J. Immunol. 131: 611- 616, 1983.

6 Berzins, T., Axelsson, B., Hammarstrom, M-L., et al.: Studies on the role of lymphocyte function-associated antigen 1 (LFA-1) in T cell activation. Scand. J. Immunol. 27: 7-16, 1988.

7 DeFougerolles, A.R., Stacker, S.A., Schwating, R., et al.: Characterization of ICAM-2 and evidence for a third counter-receptor for LFA-1. J. Exp. Med. 174: 232-237, 1991.

8 DeFougerolles, A.R., Springer, T.A.: Intercellular adhesion molecule 3, third adhesion counter-receptor for lymphocyte function-associated molecule 1 on resting lymphocytes. J. Exp. Med. 175: 185-190, 1992.

9 Kelleher, D., Murphy, A., Cullen, D.: Leukocyte function-associated antigen 1 (LFA-1) is a signaling molecule for cytoskeletal changes in a human T cell line. Eur. J. Immunol. 20: 2351-2354, 1986.

10 Kupffer, A, Singer, S.J.: The specific interaction of helper T cells and antigen-presenting B cells. IV. Membrane and cytoskeletal reorganizations in the bound T cell as a function of antigen dose. J. Exp. Med. 170: 1697-713, 1989.

11 Ikewaki, N., Yamada, A., Sonoda, A., et al.: A novel monoclonal antibody mNI-58A against the α-chain of leukocyte function-associated antigen-1 (LFA-1) blocks the homotypic cell aggregation and actively regulates morphological changes in the phorbol myristate acetate (PMA)-activated human monocyte-like cell line, U937. Tissue Antigens 48: 161-173, 1996.

12 Yagita,M., Huang,C.L., Umehara,H., et al.: A novel

natural killer cell line (KHYG-1) from a patient with aggressive natural killer cell leukemia carrying a p53 point mutation. Leukemia 14: 922-930, 2000.

13 Ikewaki, N., Inoko, H.: Development and characterization of a novel monoclonal antibody (mNI-11) that induces cell adhesion of the LPS-stimulated human monocyte-like cell line U937. J. Leukoc. Biol. 59: 697-708, 1996.

14 Rothlein, R., Springer, A.: The requirement for lymphocyte function-associated antigen-1 in homotypic leukocyte adhesion stimulated by phorbol ester. J. Exp. Med.163: 1132-1149, 1986.

15 Melero, I., Balboa, M.A., Alonso, J.L., et al.: Signaling through the LFA-1 leukocyte integrin actively regulates intercellular adhesion and tumor necrosis factor-α production in natural killer cells. Eur. J. Immunol. 23: 1859-1865, 1993.

16 Tenner, A.J.: C1q receptors regulating specific functions of phagocytic cells. Immunobiology 199: 250-264, 1998.

17 McGreal, E.P., Ikewaki, N., Akatsu, H., et al.: Human C1qRp is identical with CD93 and the mNI-11 antigen but does not bind C1q. J. Immunol. 168: 5222-5232, 2002.

18 Norsworthy, P.J., Fossati-Jimack, L., Cortes-Hernandez, J., et al.: Murine CD93 (C1qRp) contributes to the removal of apoptotic cells in vivo but is not required for C1q-mediated enhancement of phagocytosis. J. Immunol. 172: 3406-3414, 2004.

19 Poole, A.W., Pula, G., Hers, I., et al.: PKC-interactingproteins: from function to pharmacology. Trends Pharmacol. Sci. 25: 528-535, 2004.

20 Spitaler, M., Cantrell, D.A.: Protein kinase C and beyond. Nat. Immunol. 5: 785-790, 2004 .

21 Ni, H., Ergin, M., Tibudan, S.S., et al.: Protein kinase C-delta is commonly expressed in multiple myeloma cells and its downregulation by rottlerin causes apoptosis. Br. J. Haematol. 121: 849-856, 2003.

22 Tillman, D.M., Izeradjene, K., Szucs, K.S., et al.: Rottlerin sensitizes colon carcinoma cells to tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis via uncoupling of the mitochondria independent of protein kinase C. Cancer Res. 63: 5118-5125, 2003.

23 Ikewaki, N., Jerzy, K.K., Inoko, H.: Regulation of CD93 cell surface expression by protein kinase C isoenzymes. Microbiol. Immunol. 50: 93-103, 2006.

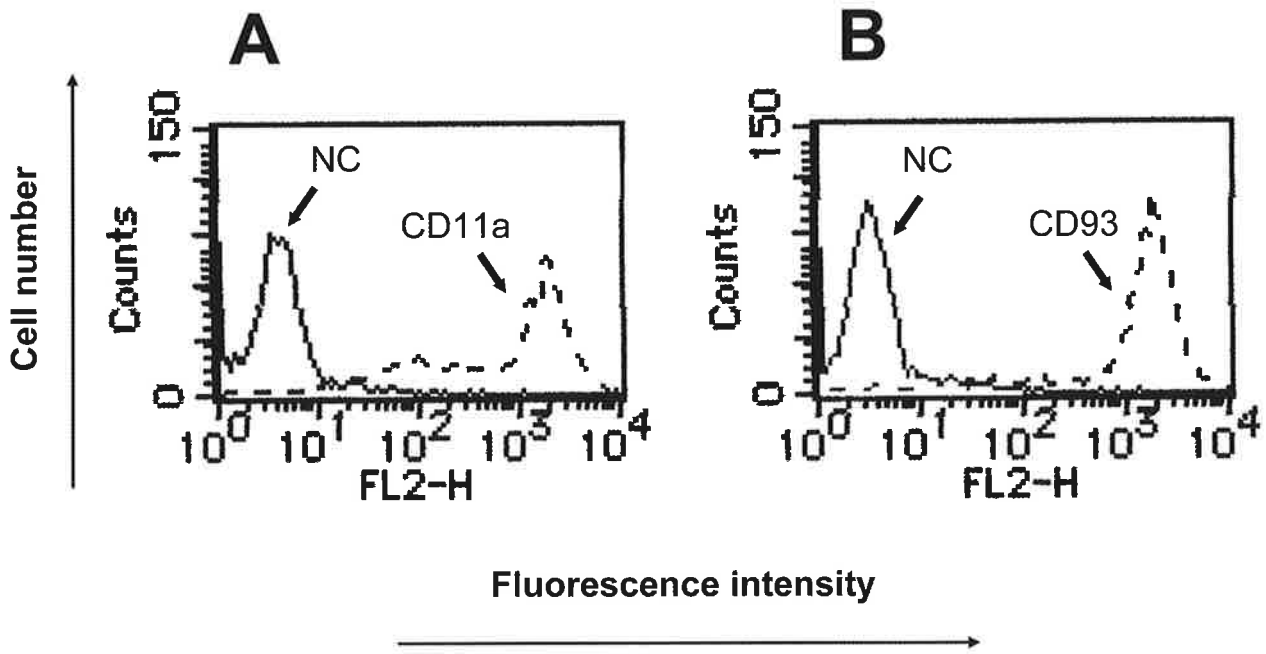

Figure 1. Expression patterns of CD11a and CD93 on KHYG-1 cells. The KHYG-1 cells were incubated with an optimal concentration of CD11a mAb (mNI-58A) or CD93 mAb (mNI-11) for 40 min at 4℃. After washing with PBS, the cells were incubated with an FITC-conjugated goat anti-mouse IgG for 20 min at 4℃. The percentages of cells that stained positively for CD11a mAb (panel A) or CD93 mAb (panel B) were analyzed using a FACScan system. Negative controls (NCs) were stained with isotype-matched normal mouse IgG1. The experiments were repeated three times.

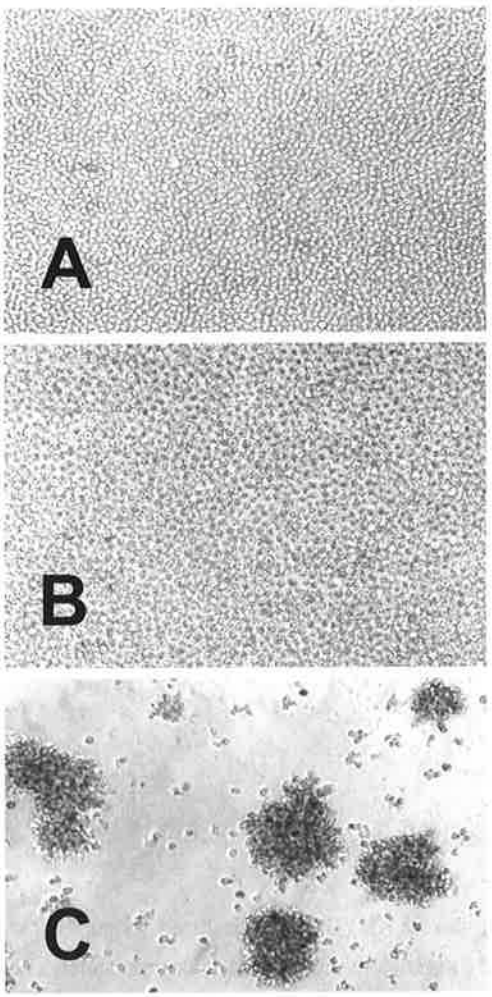

Figure 2. Induction of homotypic cell aggregation in KHYG-1 cells by mNI-58A. KHGY-1 cells were cultured in complete medium with isotype-matched normal mouse IgG1 (5 μg/mL; panel A), with mNI-11 (CD93 mAb; 5 μg/mL; panel B) or with mNI-58A (CD11a mAb; 5 μg/mL; panel C) for 18 hrs at 37℃. Morphological changes in the cells (100x) were photographed using an Olympus camera body under a phase-contrast microscope. The experiments were repeated five times.

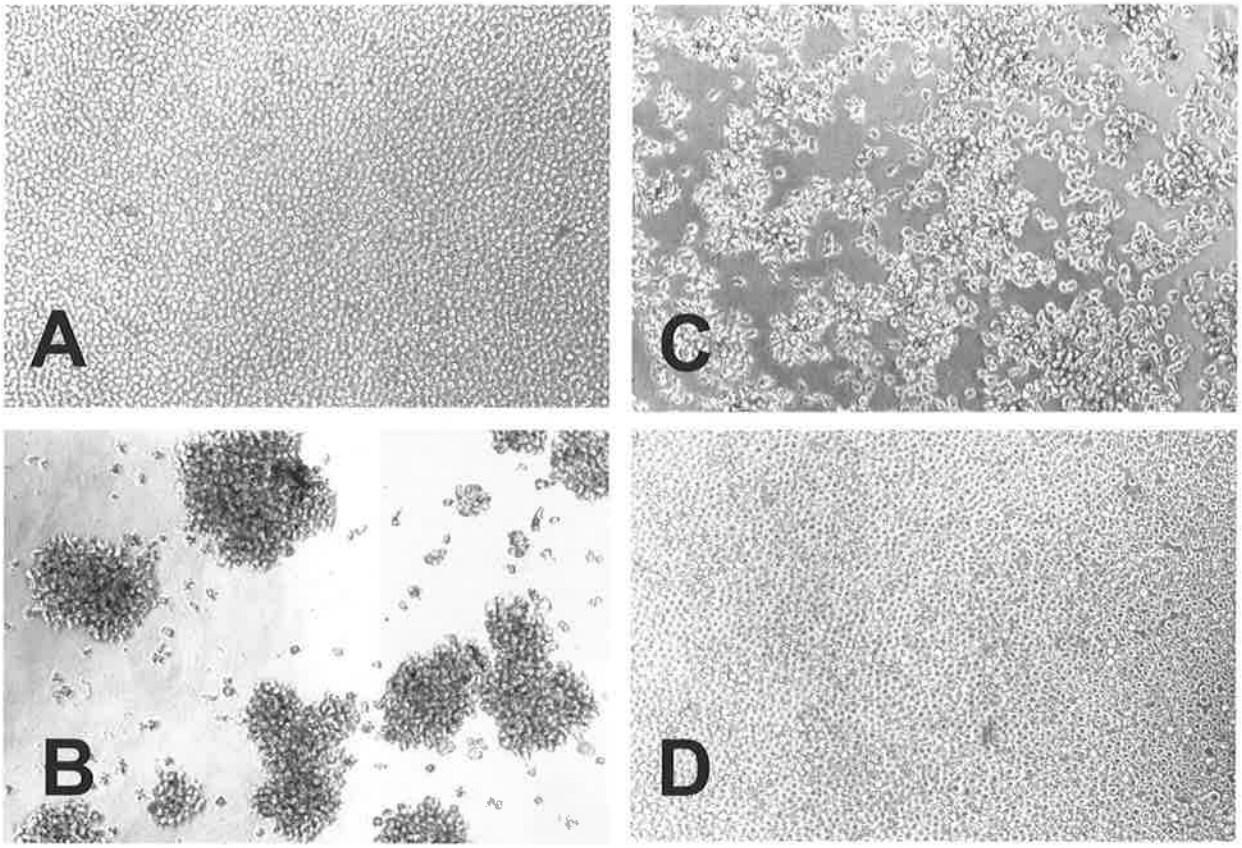

Figure 3. The homotypic cell aggregation of KHYG-1 cells induced by mNI-58A was blocked in the presence of PKC inhibitors. KHGY-1 cells were cultured in complete medium with isotype-matched normal mouse IgG1 (5 μg/mL; panel A) or with mNI-58A (CD11a mAb; 5 μg/mL; panel B) in the presence of the cPKC inhibitor Go6976 (1.5 μM; panel C) or the nPKC inhibitor Rottlerin (1.5 μM; panel D) for 18 hrs at 37℃. Morphological changes in the cells (100x) were photographed using an Olympus camera body under a phase-contrast microscope. The experiments were repeated five times.

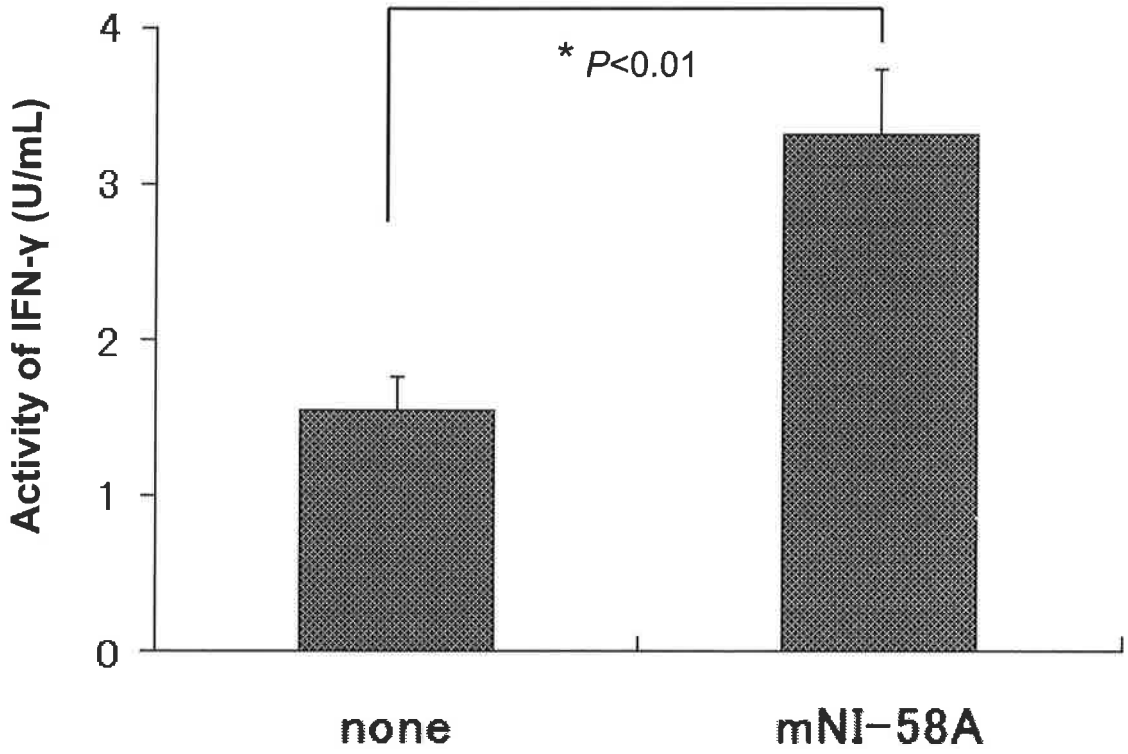

Figure 4. Promotion of IFN-γ production from KHYG-1 cells cultured with mNI-58A. Detection of IFN-γ in the culture supernatants from KHYG-1 cells cultured with or without mNI-58A (5μg/mL) for 18 hrs was performed using an EIA kit. The experiments were repeated three times. *P<0.01 (None vs. mNI-58A).

CD11aモノクローナル抗体(mNI-58A)のヒトNK様細胞株(KHYG-1)に対する細胞間凝集の誘導とインターフェロンγの産生増強

池脇信直　玉内秀一*

九州保健福祉大学薬学部動物生命薬科学科・高梁学園免疫研究所
〒882-8508　宮崎県延岡市吉野町1714-1
*北里大学医学部微生物学
〒228-8555　神奈川県相模原市北里1-15-1

要旨

我々が開発したCD11a(LFA-1α)モノクローナル抗体(mNI-58A)のヒトNK様細胞株(KHYG-1)に与える影響を検討したところ、mNI-58A抗体はKHYG-1細胞に対して細胞間凝集を特異的に誘導した。この細胞間凝集反応は、conventional protein kinase C (cPKC) 抑制剤であるGo6976とnovel protein kinase C (nPKC) 抑制剤であるRottlerinでブロックされ、その効果はRottlerinの方がGo6976より強かった。また、mNI-58A抗体はKHYG-1細胞にインターフェロンγの産生能も増強させた。以上の結果は、mNI-58A抗体がヒト免疫応答におけるNK細胞の機能を解析する上で非常に有益な抗体であることを示唆している。

キーワード：CD11a、インターフェロンγ、KHYG-1細胞、 mNI-58A
2008.11.26受理