

報 文

Natural Killer and Lymphokine-Activated Killer Activities against Autologous Myeloma Cells in Multiple Myeloma Patients, and Essential Role of Adhesion Molecule CD54 on the Killing

Fumio KOMATSU*¹, Mitsuyo KIMURA*¹ and Michiko KAJIWARA*²

Abstract

Natural killer (NK) and lymphokine-activated killer (LAK) activities in 4 multiple myeloma patients were investigated. The surface markers of the myeloma cells were assayed by a flow cytometry method. The LAK cells were generated from their peripheral blood mononuclear cells. The myeloma cells expressed 2 types of surface marker: CD38+CD56+CD49e+MPC-1+ (mature type), and CD38+CD56+CD49e-MPC-1- (immature type). In a stable state of the disease, the myeloma cells showed the mature type and expressed CD54. The NK and LAK activities of the patients were preserved, and LAK cells killed the autologous myeloma cells. Whereas, the myeloma cells in advanced stage or serious illness showed the immature type and expressed no CD54. These patients had no NK but LAK activity. However, their LAK cells could not kill the autologous myeloma cells. Results of the blocking test indicated that CD54 plays a main role in the killing, and that the resistance of the immature type to LAK-lysis is due to the CD54 depletion. It is suggestive that the impairment of the killer activity in the advanced stage or serious illness may have a relationship with the progression of the disease.

Introduction

Multiple myeloma (MM) is a clonal expansion of malignant plasma cells in the bone marrow and peripheral blood. Clinical observations suggest that the immune system plays a role in the regulation of this disease^{1,2}. T cells appear to be the element of the immune system that plays the main role in

regulation of MM³. Natural killer (NK) cells also appear to be important mediators in regulation of MM^{4,5}. However, the specific roles of NK cells in anti-myeloma effects are unclear, because there are several subtypes of this disease⁶. In the present study, we investigated the killer activities of NK cells and lymphokine-activated killer (LAK) cells in MM patients. The LAK cells were generated from peripheral blood mononuclear cells (PBMCs) of MM patients by incubating the PBMCs with interleukin-2 (IL-2).

The myeloma cells in this study were identified using a flow cytometry method. Some studies⁶⁻⁹ indicate that myeloma cells express the markers CD38, CD56 and CD138, but not CD19, on the cell surface. Kawano *et al.*^{6,7} classified myeloma cells into 2 types: mature type (CD38+CD56+CD49e+MPC-1+), and immature type (CD38+CD56+CD49e-MPC-1-). In clinical study, however, other marker combinations, including CD56-CD19+, CD56-CD19-, CD49e-MPC-1+ and CD49e+MPC-1-, are frequently found. These markers may indicate different stages of MM. We previously examined NK and LAK activities against autologous myeloma cells, and did not obtain consistent results, because these activities differed among patterns of the disease. The patients in the present study were clearly classifiable into mature and immature types. Consequently, we were able to compare killer activities between these 2 types.

For comparisons between effector cells, we used the NK cell line NK-92, which was established from cells obtained from a patient with non-Hodgkin's lymphoma¹⁰. NK-92 exhibits high killer activity against several tumor cell types^{11,12}, because NK-92

*¹ Laboratory of Clinical Physiology, Kagawa Nutrition University.

*² Blood Transfusion Service, Tokyo Medical and Dental University.

cells have no natural killer cell inhibitory receptors (KIRs)¹³, and its cytolytic effects are not affected by inhibitory signals from major histocompatibility complex (MHC) class I molecules of the target cells. NK-92 cells cannot kill CD54-deficient cells¹⁴. CD54 is a universal adhesion molecule, and plays an important role in NK- and LAK-mediated cytotoxicity^{15, 16}. We also examined the participation of CD54 in NK- and LAK-mediated cytotoxicity of myeloma cells.

Materials and Methods

Patients:

The subjects were 4 MM patients admitted to the Tokyo Medical and Dental University Hospital at nearly the same time in order to undergo high-dose chemotherapy and autologous peripheral blood stem cell (PBSC) transplantation.

In case 1 (Niwa, 49-year-old male), the patient was diagnosed as MM 5 years before admission, based on lumbago and hypergammaglobulinemia. He had been treated with single drugs and combinations of several drugs, including melphalan, prednisolone, vincristine and interferon- α . During the last 4 years before admission, serum IgD ranged from 2,200 to 2,500 mg/dl, and 30 to 35% of bone marrow mononuclear cells were myeloma cells. One year before admission, 31% and 37% of mononuclear cells in the peripheral blood and bone marrow, respectively, were CD38-positive. The disease remained in a stable condition during most of the 5 years before admission. However, 6 months before admission, the patient developed renal dysfunction and rapidly developed anemia. At admission, 68% of the PBMCs were CD38-positive, and the disease was evaluated as being in the advanced stage. The patient was admitted in order to undergo PBSC collection. PBMCs were obtained from the patient in the stable period (Niwa-1) and in the advanced stage (Niwa-2), and both samples were analyzed. The Niwa-1 sample was obtained and analyzed 1 year before admission.

In case 2 (Tak, 46-year-old male), the patient had exhibited hypergammaglobulinemia for 6 years before admission, and his serum IgG was 3,000 to 3,100 mg/dl at admission. He had been treated with melphalan, prednisolone and interferon- α . His disease had remained in a stable condition, but a mediastinum tumor (plasmacytoma) was detected 4 months before admission. He was admitted in order to undergo PBSC collection. At admission,

42% of his bone marrow cells were CD38-positive.

In case 3 (Noda, 51-year-old male), the patient was admitted because of pneumonia. He had suffered from lumbago for 2 years before admission. The patient had anemia, hypergammaglobulinemia and Bence-Jones protein in the urine. The level of serum IgA was 1,800 mg/dl. Analysis showed that 36% of the bone marrow cells were myeloma cells. The patient's MM was in a stable condition at admission. However, the patient died of acute renal failure due to a large renal tumor.

In case 4 (Miy, 60-year-old male) the patient was diagnosed with MM 3 months before admission, based on observation of anemia, renal dysfunction and hypergammaglobulinemia (IgG 4,400 mg/dl). Examination revealed that 80% of bone marrow cells were CD38-positive. The patient was evaluated as being very severely ill, and he was admitted in order to undergo PBSC collection.

Separation of PBMCs:

Informed consent was obtained from all patients before the determination. PBMCs were separated from the heparinized peripheral blood of the 4 MM patients and 6 healthy volunteers (controls) by Ficoll-Hyperque gradient centrifugation. The red blood cells and granulocytes were discarded by this centrifugation. The remaining cells were incubated in a plastic culture flask for 1 hr at 37°C. After incubation, adherent cells (monocytes) were removed.

Determination of Cell Surface Markers:

Cell surface markers were assayed using a flow cytometry method. The following commercially available fluorescein isothiocyanate- or phycoerythrin-conjugated specific monoclonal antibodies (mAbs) were used: CD16, CD19, CD38, CD49e, CD56 and CD138 (Immunotech SA, Marseillu, France); CD2, CD18, CD54 and CD58 (DAKO A/S Produkt, Glostrup, Denmark); and MPC-1 (donated by Otsuka Tokyo Assay Laboratories Co. Ltd, Tokyo, Japan). MPC-1 recognizes an antigen on mature myeloma cells and normal plasma cells⁸. For the assay of HLA-monomorphic determinant, the mAb W6/32 (DAKO A/S Produkt) was used. For the assays of HLA-polymorphic determinants, anti-HLA mAbs (One Lambda Inc., Los Angeles, CA, USA) were used. These assays were performed using a dual staining technique. The PBMCs were incubated with these mAbs for 30 min at 4°C. Then, after washing to remove unbound mAbs, the percentage

of fluorescence-stained cells and fluorescein intensity (mean channel) were determined using the Ortho Cytoron (Ortho Diagnostic Systems, Raritan, NJ, USA).

Effector Cells:

- 1) NK cells: For the assay of NK cell-mediated cytotoxicity, the above-described PBMCs were used as the effector cells.
- 2) LAK cells: The PBMCs were cultured in a medium (RPMI 1,640 + 10% fetal calf serum) supplemented with 400 IU/ml recombinant IL-2 (Shionogi Pharmaceutical Co. Ltd., Osaka, Japan). LAK cells were generated by culturing these PBMCs for 7 days at 37°C.
- 3) NK-92: For comparison between effector cells, the NK cell line NK-92 was used. NK-92 was obtained from Immune Medicine Inc. (Vancouver, BC, Canada), and was maintained by continuous culturing in Myelo Cult H5100 medium (Stem Cell Technologies Inc., Vancouver, BC, Canada) supplemented with 20 IU/ml recombinant IL-2 and 10^{-6} M hydrocortisone.

Target Cells:

For the assay of NK and LAK activities, a myeloid leukemia cell line K562 and a lymphoblastoid cell line Raji (American Type Culture Collection, Rockville, MD, USA) were used as target cells, respectively. These cell lines were maintained by culturing in medium (RPMI 1,640 + 10% fetal calf serum). For the assay of NK and LAK activities against autologous myeloma cells, purified CD38-positive cells were used as target cells. The purification of CD38-positive cells from CD38-negative cells was performed by negative selection. CD16-, CD4- and CD8-positive cells were discarded using monoclonal antibody-coated Dynabeads (DynaL. A.S., Oslo, Norway). After the purification, the mature type of CD56+CD49e+MPC-1+ and the immature type of CD56+CD49e-MPC-1- on the surface of target cells were confirmed.

Determination of NK and LAK Activities and NK-92-Mediated Cytotoxicity:

NK and LAK activities and NK-92-mediated cytotoxicity were assayed using a 4h- ^{51}Cr -release assay. The target cells were labeled with $\text{Na}_2^{51}\text{CrO}_4$ for 1 hour at 37°C and washed twice with PBS. Then, the target cells (T), alone or with the effector cells (E), were transferred to a microtiter plate and

incubated for 4 hours at 37°C. The E:T ratio was adjusted to 10:1. Next, the radioactivity of supernatants was determined using a gamma counter. Maximum CPM was produced by the target cells incubated with 1% Nonidet P40. Spontaneous CPM was produced after incubation of the target cells in medium alone. Experimental CPM was produced from the mixture of target cells and effector cells. The ^{51}Cr -release rate (killer activity) was calculated as follows: $[(\text{Experimental CPM} - \text{Spontaneous CPM}) / (\text{Maximum CPM} - \text{Spontaneous CPM})] \times 100 (\%)$.

Assay for Disappearance of CD38-Positive Cells in Culturing:

An assay was performed to examine whether CD38-positive cells would disappear in culturing. The PBMCs were suspended in medium (RPMI 1,640 + 10% fetal calf serum) supplemented with 400 IU/ml recombinant IL-2. After culturing for 5 days, the percentage of remaining CD38-positive cells was counted.

Blocking Tests:

Blocking tests against adhesion molecules CD54 and CD58 on the target cells were performed. The ^{51}Cr -labelled target cells were incubated with specific mAbs for 30 min at 4°C. Then, they were incubated with the effector cells, and changes in ^{51}Cr -release rates were determined as described above. Anti-CD54 and anti-CD58 mAbs (DAKO A/S Produkt) were used to block CD54 and CD58, respectively.

All assays were performed in triplicate, and the averages of the results were calculated. For the healthy volunteers (controls), the results are shown as mean (M) $\pm$ SD. Differences were considered significant if the experimental sample produced a result beyond the range of the controls (M $\pm$ 2SD).

Results

1. Surface Markers of PBMCs in MM Patients

Surface markers of PBMCs in the 4 MM patients are shown in Table 1. Niw remained in a stable state of the disease for 4 years, and then his disease changed to an advanced stage. The label "Niw-1" is used to indicate patient Niw in the stable condition, and "Niw-2" is used to indicate patient Niw in the advanced stage. Tak remained in a stable state after admission, despite the development of a plasmacytoma in the mediastinum. Nod also

Table 1. Surface Markers of PBMCs in Multiple Myeloma Patients

	Niw-1	Niw-2	Tak	Nod	Miy	V-PBMC	
CD38+CD56+CD49e+MPC-1+	31	0	44	35	0	0	%
CD38+CD56+CD49e-MPC-1-	0	68	0	0	79	0	
CD16	14	11	13	11	10	12.5±1.8	
CD56	47	80	59	48	90	18.3±2.1	
W6/32	100	100	100	100	100	100.0±0.0	
CD54	31	0	44	35	0	0.8±0.1	
CD58	14	0	8	16	0	8.7±0.6	
CD18	68	22	64	60	18	98.1±1.8	
CD2	64	17	60	56	15	91.3±8.4	

Surface markers were assayed using the Ortho Cytoron.

Samples for Niw-1, Tak and Nod were obtained from a stable state of the disease.

Samples for Niw-2 and Miy were obtained at the advanced stage and serious illness, respectively.

V-PBMC: healthy volunteers (n=6, M±SD)

remained in a stable state of MM, but he died from acute renal failure. In contrast, Miy developed serious illness at onset of MM. The percentage of CD38-positive cells in the PBMCs differed widely among the 4 patients. The CD38-positive cells were also positive for CD56 and CD138, but were negative for CD19. Niw-1, Tak and Nod exhibited cells with the phenotype CD38+CD56+CD49e+MPC-1+, which was classified as the mature type by Kawano *et al.*^{6, 7)}. Niw-2 and Miy exhibited cells with the phenotype CD38+CD56+CD49e-MPC-1-, which has been classified as the immature type. The myeloma cells of Niw rapidly changed from the mature type to the immature type, corresponding to the disease course.

Table 1 also shows the results of assays for the markers CD16, CD56, W6/32, CD54, CD58, CD18 and CD2. In order to determine the percentage of NK cells, the PBMCs were stained with anti-CD16 and anti-CD56 mAbs. CD16 was not expressed on the CD38-positive cells, whereas CD56 was expressed on both CD38-positive cells and CD16-positive cells. This indicates that CD16 is a specific marker of NK cells, and that CD56 is not. The percentages of CD16-positive cells in the 4 patients were considered roughly equal to those of the 6 healthy volunteers (V-PBMCs), because their results were within the range of $M \pm 2SD$.

To assay for expression of HLA class I molecules, PBMCs were stained for the HLA-monomorphic determinant W6/32. In all 4 MM patients, 100% of PBMCs were positive for W6/32, although the fluorescein intensity of the immature cells was weaker than that of the mature cells. Also, 100% of these PBMCs expressed the HLA-polymorphic determinants (data not shown). It indicates that

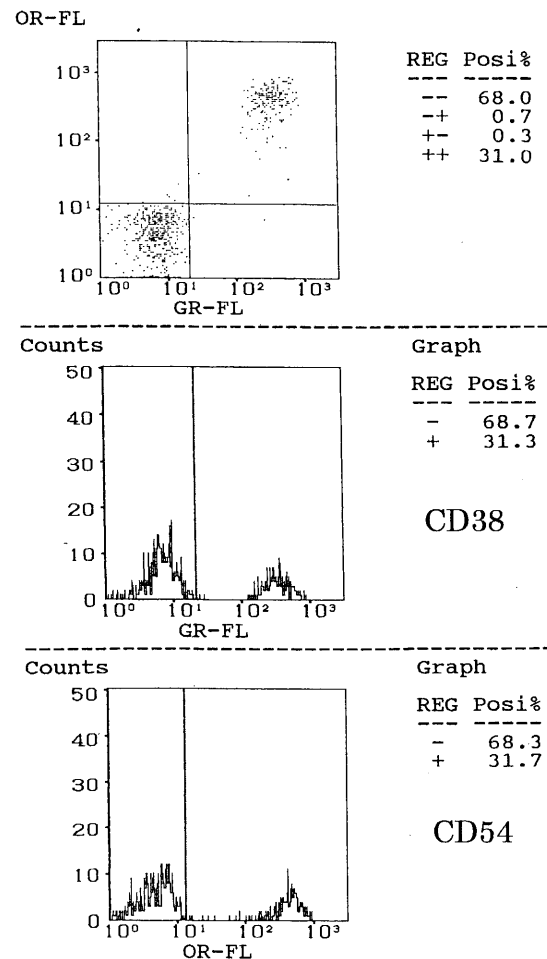


Fig. 1. Dual staining of Niw-1 PBMCs with anti-CD38 and anti-CD54 mAbs. The PBMCs were stained with these mAbs for 30 min at 4°C. Then, after washing, the percentages of fluorescence-stained cells were determined using the Ortho Cytoron. The CD54-positive cells entirely overlapped with the CD38-positive cells.

the myeloma cells did not lose expression of HLA class I molecules.

To assay expression of the adhesion molecules

Table 2. NK and LAK Activities of PBMCs in Multiple Myeloma Patients

	Niw-1		Niw-2		Tak		Nod		Miy		V-PBMC	
	NK	LAK	NK	LAK	NK	LAK	NK	LAK	NK	LAK	NK	LAK
K562	35.8	—	0.4	—	33.7	—	29.8	—	0.6	—	56.4±4.2	—
Raji	—	38.4	—	24.7	—	34.1	—	30.4	—	21.7	—	62.5±5.3

LAK cells were generated from the PBMCs of the patients by stimulating with interleukin-2.

NK and LAK activities were determined by a 4h-⁵¹Cr-release assay. E:T=10:1.

V-PBMC: healthy volunteers (n=6, M±SD).

CD54 and CD58, dual staining was performed. In Niw-1, CD54-positive cells entirely overlapped with the CD38-positive cells (Fig. 1). Tak and Nod also exhibited overlapping of CD54-positive cells with CD38-positive cells. In contrast, Niw-2 and Miy did not exhibit expression of CD54 on the cell surface. The staining rate of CD58 varied among the 4 patients. CD58-positive cells did not always overlap with the CD38-positive cells. CD58 was also detected on non-myeloma cells. Some cells of the mature type were positive for CD54 and CD58 (CD54bright CD58moderate), whereas all immature type cells were negative for both of them. CD18 and CD2 did not always overlap with the CD38-positive cells. Some of the CD38-positive cells in the mature type expressed CD18 and CD2 weakly, but those in the immature type did not express either of them. While, the CD16-positive cells in all patients expressed CD18 and CD2.

2. NK and LAK Activities of PBMCs in MM Patients

Because the PBMCs of the patients and controls had roughly equal percentages of CD16-positive cells, we determined the NK activity against K562 and LAK activity against Raji. NK and LAK activities varied among the 4 patients (Table 2). Niw-1, Tak and Nod showed NK and LAK activities corresponding to about 2/3 of the values obtained for the controls. However, Niw-2 and Miy exhibited no NK activity, but exhibited a low level of LAK activity. It indicates that the killer cell function was compromised in MM patients, in particular, in the advanced stage and serious illness. However, the killer cell function was restored by culturing with IL-2.

3. Disappearance of Myeloma Cells during Incubation with IL-2

To determine whether IL-2-stimulated killer cells (LAK cells) can kill autologous myeloma cells, PBMCs from the patients (containing myeloma

Table 3. Disappearing of Myeloma Cells during Incubation with IL-2

	Preincubated	Incubated		
		IL-2(-)	IL-2(+)	
CD38+CD56+CD49e+MPC-1+				
Niw-1	31	24	0	%
Tak	44	36	0	
Nod	35	30	0	
CD38+CD56+CD49e-MPC-1-				
Niw-2	68	65	61	
Miy	80	76	72	

PBMCs were incubated in the medium with IL-2 or without IL-2 for 5 days at 37°C, and then, remaining CD38-positive cells were counted. Average results in triplicated experiments were shown.

cells) were incubated in medium supplemented with IL-2 for 5 days at 37°C, followed by counting of the percentage of remaining CD38-positive cells by the flow cytometry method. When the PBMCs of Niw-1, Tak and Nod were incubated with IL-2, there was marked disappearance of CD38-positive cells, whereas most CD38-positive cells remained after incubation without IL-2 (Table 3). This finding indicates that, in these patients, the autologous myeloma cells were killed by the LAK cells. In contrast, with Niw-2 and Miy, the CD38-positive cells did not disappear during incubation with or without IL-2.

4. NK and LAK Activities against Autologous Myeloma Cells

In order to confirm the above results, we assayed killer activity against autologous myeloma cells. As target cells, autologous CD38-positive cells were used. The autologous CD38-positive cells were purified from the PBMCs by negative selection. Purity of greater than 90 % of CD38-positive cells was obtained by this method. Niw-1, Tak and Nod exhibited weak NK-lysis and moderate LAK-lysis against autologous myeloma cells (mature type) (Table 4). By this experiment, it was confirmed that the autologous myeloma cells were killed by

Table 4. NK and LAK Activities against Autologous Myeloma Cells

	Niw-1		Niw-2		Tak		Nod		Miy		V-PBMC		
	NK	LAK	NK	LAK	NK	LAK	NK	LAK	NK	LAK	NK	LAK	
CD38+CD56+CD49e+MPC-1+	5.2	37.4	—	—	5.1	33.6	2.1	28.3	—	—	0.1±0.0	56.3±7.1 ^{a)}	%
CD38+CD56+CD49e-MPC-1-	—	—	0.0	0.3	—	—	—	—	0.0	0.7	0.3±0.1	0.8±0.1 ^{b)}	

Autologous myeloma cells were purified by depletion of CD4-, CD8- and CD16-positive cells.

V-PBMC: healthy volunteers (n=6, M±SD), ^{a)} against Tak myeloma cells, ^{b)} against Niw-2 myeloma cells.

Table 5. Blocking Tests against CD54 and CD58 of Autologous Myeloma Cells

	Niw-1	Tak	Nod	V-PBMC	NK-92
	LAK	LAK	LAK	LAK	
CD38+CD56+CD49e+MPC-1+					
Non-blocking	37.5	30.5	27.3	48.3±5.5 ^{a)}	52.1 ^{a)} %
Blocking with CD54	0.5	0.1	0.1	1.6±0.2 ^{a)}	0.0 ^{a)}
Blocking with CD58	35.8	29.7	26.2	46.5±4.3 ^{a)}	50.9 ^{a)}
Raji					
Non-blocking	38.5	34.1	30.7	62.5±5.3	63.8
Blocking with CD54	0.4	0.3	0.1	12.7±1.4	0.1
Blocking with CD58	29.6	25.8	21.4	38.6±4.2	62.6

As target cells, purified myeloma cells were used. ^{a)} Target cells were Tak myeloma cells.

V-PBMC: healthy volunteers (n=6, M±SD).

NK-92: a NK cell line.

the LAK cells.

Because the autologous myeloma cells in Niw-1, Tak and Nod were killed by NK cells (although the killing levels were weak), we assayed expression of HLA class I antigens (MHC molecules) on CD38-positive cells, because MHC molecule-depleted target cells are generally killed by NK cells. The CD38-positive cells were positive for W6/32 and HLA-polymorphic determinants, as described above. It indicates that the NK-lysis was not due to suppression of MHC molecules.

Meanwhile, Niw-2 and Miy did not exhibit NK-lysis or LAK-lysis against autologous myeloma cells (immature type), showing that killer cell function is compromised in the advanced stage and serious illness. Furthermore, it also means that the immature type cells lost the susceptibility to the killer cells, because the controls' LAK cells killed mature type cells but did not kill immature type cells.

5. Blocking Tests against CD54 and CD58 of Autologous Myeloma Cells

Because Niw-1, Tak and Nod exhibited overlapping of CD54-positive cells with CD38-positive cells, as described above, we performed a blocking test against autologous myeloma cells using anti-CD54

mAb. It was confirmed beforehand that all CD16-positive cells in these 3 patients expressed markedly high levels of CD18 and CD2. The purified autologous CD38-positive cells were used as the target cells. The cell line Raji, which expresses CD54 and CD58, was used for comparison of the target cells. The NK cell line NK-92 was used for comparison of effector cells, because NK-92 did not kill CD54-deficient target cells¹⁴⁾. Blocking using anti-CD54 mAb conspicuously suppressed the killing levels (Table 5), and also markedly decreased NK-92-lysis. A blocking test using anti-CD58 mAb was also performed, but showed no blocking effect. While, blocking tests with Niw-2 and Miy (their myeloma cells did not express CD54 or CD58) did not show any blocking effect on the killing (data not shown).

Discussion

The present patients showed 2 phenotypes of cell surface marker: CD38+CD56+CD49e+MPC-1+ (mature type) and CD38+CD56+CD49e-MPC-1- (immature type). These 2 phenotypes are representative of myeloma cells^{6,7)}. However, in clinical examinations, several subtypes have also been found. In patients with these subtypes, it is very difficult to accurately assess NK and LAK activities. In this study, Niw-1, Tak and Nod (from whom PBMCs were obtained

in a stable period) exhibited the mature type, and Niw-2 and Miy (from whom PBMCs were obtained in advanced stage or serious condition) exhibited the immature type. Niw had been in a stable state for 4 years, but his disease then rapidly changed to the advanced stage. Corresponding to his disease course, his myeloma cells changed from the mature type to the immature type. Generally, cells of the immature type appear in the serious state of this disease.

CD16 and CD56 are thought to be markers of NK cells. However, CD56 is expressed not only on CD16-positive cells but also on CD38-positive cells. Thus, CD56-positive cells could not be conclusively identified as NK cells in the present patients, although Hall *et al.*¹⁷⁾ and King *et al.*¹⁸⁾ regard CD56-positive cells as NK cells. The present patients exhibited percentages of CD16-positive cells that were roughly equal to those obtained for controls. A study by King *et al.*¹⁸⁾ indicates that either the percentage or the absolute number of NK cells does not correlate with disease activity. It is likely that NK cell numbers may be maintained even in the advanced stage or serious condition.

In the present study, NK activity against K562 and LAK activity against Raji differed between patients and controls. Niw-1, Tak and Nod exhibited moderate NK and LAK activities. Niw-2 and Miy did not exhibit NK activity, although they exhibited a low level of LAK activity. This finding suggests that killer cell function is compromised in MM patients, particularly in the advanced stage or serious illness. Frassanito *et al.*¹⁹⁾ reported that the impairment of killer cell function may be caused by the inhibitory effect of M-component. In our patients, Niw-2 and Miy exhibited no NK activity, but low LAK activity. Killer cell function may have been inhibited by the M-component, as described by Frassanito *et al.*¹⁹⁾, because the killer cell function was restored by culturing with IL-2. Restoration of killer cell function by incubation with IL-2 has also been observed for other diseases²⁰⁾.

Also in the present study, NK and LAK activities against autologous myeloma cells were determined. Niw-1, Tak and Nod exhibited weak NK activity and moderate LAK activity against autologous myeloma cells. Meanwhile, Niw-2 and Miy did not exhibit NK or LAK activity. Concerning the NK-lysis in Niw-1, Tak and Nod, we assayed for MHC molecules on the myeloma cells. The myeloma cells expressed these determinants. Thus, the NK-

lysis was considered to be not due to the MHC molecules^{21, 22)}. However, in some studies, MHC molecule-positive myeloma cells were killed by NK cells^{5, 23)}. Zheng *et al.*²³⁾ found that autologous MM cells exhibited high susceptibility to NK-lysis, despite their high expression of MHC molecules. They speculated that the cytotoxicity towards autologous MM cells was due to innate immunity. Recently, Cerwenka, *et al.*²⁴⁾ reported that ectopic expression of retinoic acid early inducible-1 gene (*RAE-1*) permits NK cell-mediated rejection of MHC molecule-bearing tumor cells *in vivo*. They found that, when tumor cells were transfected with *RAE-1*, the tumor cells were killed by NK cells, despite expression of MHC molecules. Thus, they concluded that expression of *RAE-1* on tumor cells is important for NK-lysis. In the present study, we were unable to elucidate the mechanism of NK-lysis against MHC molecule-positive autologous myeloma cells. Further study is needed to elucidate this type of killing.

Now, we should consider the reason why Niw-2 and Miy did not exhibit LAK-lysis against autologous myeloma cells but did exhibit LAK-lysis against the allogeneic cell line Raji. We speculate that the resistance to the LAK-lysis is due to abnormality of the target cells. Therefore, we assayed for the adhesion molecules CD54 and CD58 on the cell surface. These adhesion molecules stimulate the costimulatory signals of cell killing^{15, 16)}. In the present study, the mature type expressed high level of CD54, but the immature type did not express. Accordingly, we speculated that the lack of susceptibility of the immature type to LAK-lysis is due to depletion of this adhesion molecule. This speculation is supported by the experiment using the NK cell line NK-92. We previously found that NK-92 did not kill CD54⁻CD58⁺ target cells^{13, 14)}. In the present study, NK-92 killed the mature type but not the immature type. It is conceivable that NK-92 can kill the mature type via the CD18/CD54 co-stimulatory signal. Furthermore, we performed a blocking test against CD54, which revealed that the anti-CD54 treatment decreased the killing. These results suggest that CD54 plays a main role in the killing, and that the resistance of Niw-2 and Miy to LAK-lysis is due to CD54-depletion. The killer cells may be able to discriminate between myeloma cells and normal mononuclear cells based on the presence of this adhesion molecule. While, the result of the blocking test indicated that CD58 plays no important role

in the present patients.

Patient-derived *ex vivo*-expanded NK cells show a strong anti-leukemia effect^{25, 26}). Studies of NK and LAK cells may lead to development of novel therapeutic regimens for the treatment of MM patients. The impairment of the killer activity in the advanced stage or serious illness may have a relationship with the progression of the disease. These results should be confirmed using further many patients, because the number of the patients in this study was not so enough.

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