Jpn. J. Cancer Res. 87, 405–411, April 1996

A Human/Mouse Chimeric Monoclonal Antibody against Intercellular Adhesion Molecule-1 for Tumor Radioimmunoimaging

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A mouse-human chimeric antibody for intercellular adhesion molecule-1 (ICAM-1) was established by using heavy chain loss mouse mutant hybridoma and human immunoglobulin expression vector. The HA58 hybridoma secreted anti-ICAM-1 monoclonal antibody (MoAb) (IgG1, κ). The gene of the mouse variable region of heavy chain was amplified and cloned by the polymerase chain reaction technique directly from the HA58 hybridoma RNA. The variable region of heavy chain was joined with an expression vector which contains human γ1 constant gene. The expression vector was transfected into heavy chain loss mutant cells HA58-7, which produced only murine immunoglobulin light chains. The resultant chimeric MoAb HA58, chHA58, retained full-binding reactivity to ICAM-1 compared with murine HA58 parental antibody. The chimeric MoAb chHA58 showed little antibody dependent cell-mediated cytotoxic activity against cultured tumor cells. Biodistribution studies with ^{99m}Tc-labeled chHA58 in nude mice bearing human gastric carcinoma JRST cells demonstrated that the tumor-blood ratio was 1.55 at 18 h after injection, when the tumors were clearly visible in gamma scintigraphy. These data suggest that chHA58 may be of practical use for radioimmunoimaging of a wide variety of tumors.

Key words: Chimeric monoclonal antibody — ICAM-1 — Radioimmunoimaging

Intercellular adhesion molecule-1 (ICAM-1), a member of the immunoglobulin supergene family with five immunoglobulin-like domains, a single transmembrane region, and a short cytoplasmic domain, has recently been characterized as a counter-receptor for leucocyte β2 integrins such as lymphocyte function associated antigen-1 (LFA-1)[1] and Mac-1,[2] as well as for CD43.[3] ICAM-1 plays an important regulatory role for cell-cell interaction not only in inflammatory, but also in malignant diseases. Prior to an inflammatory response, ICAM-1 is expressed on relatively few cell types, endothelial cells and lymphoid follicles.[4,5] Inflammatory mediators, such as tumor necrosis factor-α, interleukin 1β and interferon γ, up-regulate ICAM-1 expression on the surface of many cell types including cells of myeloid, fibroblastic, epithelial, and endothelial origin.[6,7] Indeed, it has been immunohistochemically demonstrated that a wide variety of tumor cells have higher expression levels of ICAM-1 than normal tissues.[8,9] Thus, tumor tissues would be a good target for radioimmunoimaging using anti-ICAM-1 antibodies.

The clinical applications of murine monoclonal antibodies (MoAbs) for diagnosis and therapy in human cancer have been a very active area of research.[10] Clinical trials have been conducted with murine MoAb alone or conjugated to isotopes, toxins, or drugs. But there are various problems in clinical application. The major limitation in the utility of murine MoAbs lies in the human anti-mouse antibody (HAMA) response which is usually directed against the immunoglobulin constant regions, although an appreciable anti-variable regions response is often detected.[11] Recent advances in gene technology have made it possible to construct a mouse-human chimeric antibody,[12] which has a longer circulation time than a parental mouse MoAb.[13,14]

We have developed a mouse-human chimeric monoclonal antibody against ICAM-1, designated as chHA58, by transfecting an expression vector containing the V_H cDNA of mouse MoAb HA58 and the human γ1 constant region into heavy chain loss mutant cells derived from HA58 hybridoma. The chimeric MoAb chHA58 showed specific binding affinity to ICAM-1 and a clear tumor uptake in tumor-bearing nude mice when labeled with ^{99m}Tc.

MATERIALS AND METHODS

Cell lines The MoAb from mouse hybridoma cell line HA58 (IgG1, κ) recognizes the ICAM-1.[8] HA58-7 is a heavy chain loss mutant cell line subcloned from HA58 and it produces mouse κ light chain. HA58-7 was used for transfection of the chimeric gene. A Sezary syndrome cell line Sez627c and other cell lines used in this study were maintained in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS), 2 m*M* L-glutamine and gentamycin sulfate (25 mg/ml).

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PCR-cloning of the HA58 heavy chain V region cDNA The oligonucleotides were designed using the data base of Kabat *et al.*[15] The 5′ primer was constructed from the information available on the conserved sequence of the leader regions. The 5′ end primer to heavy chain used in this experiment was 5′-CATCGATATGGGATGGAG-CTGGATCTTTC-3′, containing a *Cla*I site. The 3′ primer to the IgG1 heavy chain was 5′-CGGTACCG-GTGTCGACTTACCTGAGGAGACTG-3′, containing a *Kpn*I site. Total cellular RNA was isolated from the HA58 hybridoma cells by the guanidine isothiocyanate method as described by Davis *et al.*[16] Amplification of a segment of the mRNA was carried out by polymerase chain reaction (PCR) according to the method of Kawasaki *et al.*[17] The amplified PCR fragments of DNA were ligated into Bluescript (Stratagene, CA), and two cDNA clones were directly sequenced for comparison to detect nucleotide misincorporation based on PCR by the dideoxy chain termination method using a Sequenase kit (USB, Cleveland, OH).

Construction of expression vector and transfection into heavy chain loss mutant cells The expression vector mpSV2neo-EP1-Cγ1 was originally derived from mpSV2neo-EP1-V-Cγ1 vector, which was kindly donated by Dr. Hozumi,[12] and contains the 0.3 kb immunoglobulin heavy chain promoter fragment, the 1.5 kb immunoglobulin heavy chain enhancer fragment and the 3.2 kb human IgG genomic Cγ1 fragment and neo gene as a selection marker. The 0.4 kb HA58 heavy chain V region cDNA fragment was ligated into the *Cla*I and *Kpn*I sites of mpSV2neo-EP1-Cγ1 vector, and the expression vector mpSV2neo-EP1-Cγ1 was constructed.

The chimeric heavy chain expression vector was transfected into heavy chain loss mutant HA58-7 cells by electroporation.[18] The human immunoglobulin expression vector mpSV2neo-EP1-VHA58-Cγ1 was transfected and stable transformants were selected in the presence of G418 at 1.0 mg/ml. After 14 days, the growth supernatants were screened for human γ chain expression and binding activity to ICAM-1 using a binding radioimmunoassay. The cells with positive human γ chain expression and positive binding to ICAM-1 were subcloned and expanded on a large scale for purification. A clone of the stable transformant containing the chimeric gene was established and termed chHA58.

Purification and radiolabeling of MoAbs The mouse MoAb HA58 and the chimeric MoAb chHA58 were purified from ascites fluid by chromatography on Protein A-Sepharose (Pharmacia, Uppsala, Sweden).[19] These antibodies were radiolabeled with ^{125}I by means of the chloramine T method for binding and competition assays.[20]

^{99m}Tc-labeling for biodistribution and imaging experiments was performed according to the method reported previously.[21] Purified chHA58 at 1 mg/ml in 0.05 *M* phosphate-buffered saline, pH 7.5, was reduced with 2-mercaptoethanol (2-ME) at a 2-ME/MoAb ratio of 10000:1 at room temperature for 30 min. The reduced antibodies were then purified by gel chromatography using a G-25M Sephadex column, PD-10 (Pharmacia). The protein fraction was separated into 0.5 mg aliquots. For ^{99m}Tc-labeling, 50 μl of hydroxymethylene diphosphonate (HMDP) (Clearbone kit, Nihon Mediphysics Co., Ltd., Nishinomiya) solution reconstituted with 5 ml of 0.9 % sodium chloride was added to 0.5 mg of reduced antibody. Antibody-HMDP mixture was then incubated with 740 MBq of ^{99m}Tc pertechnetate from a ^{99}Mo/^{99m}Tc generator (Dinatec, Dainabot Co., Ltd., Tokyo) for 10 min.

Binding assays to cultured cells Indirect binding assay was performed as follows: 100 μl of MoAb supernatant was incubated with cultured Sezary syndrome Sez627c cells (1×10^5) for 2 h at 4°C. After washing, 100 μl of ^{125}I-labeled anti-mouse IgG-Fc antibody or anti-human IgG1-Fc antibodies was added to each well and the plate was incubated for 2 h at 4°C. After 5 washings, the wells were cut out and radioactivity was counted in a γ-counter. For direct binding assay, 100 μl of ^{125}I-labeled MoAb (1×10^5 cpm/well) was incubated with the cultured cell lines (1×10^5 cells) in wells for 2 h at 4°C. After 5 washings, the wells were cut out and the radioactivity was counted in a γ-counter. The association constant (K_a) of HA58 and chHA58 were measured by means of Scatchard analysis.[22] Briefly, 100 μl aliquots of serially diluted ^{125}I-labeled MoAbs (1.56×10^{-8}–4.87×10^{-10} *M*) were incubated with ICAM-1 bearing cells (Sez627c) for 1.5 h. Following 5 washings, bound radioactivity was counted. B/F and [B] were plotted to obtain K_a from the slope of the line [B, cpm of bound antibodies; F, cpm of free antibody; [B], concentration of bound antibody (*M*)].

Competition assay In order to test whether the epitopes recognized by murine MoAb HA58 and chimeric MoAb chHA58 were different, competition assay was performed. Cultured Sezary syndrome Sez627c cells were preincubated with an excess of unlabeled MoAb for 1 h at 4°C. After incubation for 1 h, bound radioactivity was counted. Results were calculated as % blocking, compared to negative control.

Antibody-dependent cellular cytotoxicity (ADCC) The target cells (Sez627c and JRST) for the ADCC assay were labeled with 100 μCi of ^{51}Cr-sodium chromate (Amersham, Buckinghamshire, UK) for 1 h at 37°C in RPMI-1640 medium. Human peripheral blood mononuclear cells used as effector cells were obtained from a healthy donor and separated by using lymphocyte separation medium (Ficoll-Paque, Sigma, St. Louis, MO). Target cells (1×10^4, 50 μl) and effector cells (100 μl) at the effector/target (E/T) ratio of 50 were added in

triplicate to round-bottomed 96-well microtiter plate with 100 μl of the purified chimeric or mouse MoAb, and incubated for 8 h at 37°C. After incubation, 100 μl of the supernatant from each well was collected and counted in a γ-counter.

Biodistribution of ^{99m}Tc-labeled chHA58 and imaging of tumors in nude mice Athymic nude mice (Balb/c, nu/nu) bearing JRST human gastric cancer cells were used for studying the biodistribution of radiolabeled chHA58. Average weight of the tumors was 0.46 g at 2 weeks after subcutaneous inoculation of JRST (1×10^7/animals) into a rear flank. The mice were injected intravenously with approximately 400 ng of ^{99m}Tc-labeled chHA58. At 3 or 18 h post-injection, the mice were anesthetized and killed, then the weight and radioactivity of major organs were measured. Biodistribution was presented as percentage of the injected dose per gram of organ normalized to a 20 g body weight. Four to five mice were examined in each group. Scintigrams of tumor xenografts were obtained with a gamma camera 18 h after injection of a high dose of ^{99m}Tc into mice with large tumors.

RESULTS

Nucleotide sequence of the V_H region of MoAb HA58 The 0.4 kb amplified PCR fragment from the RNA of the HA58 hybridoma cells was ligated into Bluescript, and then sequenced by the dideoxy chain termination method. The nucleotide and deduced amino acid sequences of the V_H region of MoAb HA58 are shown in Fig. 1. The region was assigned to subgroup II(A) according to Kabat *et al.*[15] Comparison of the sequence with those of other members of the same group showed that a stretch of six amino acids from the end of FR3 to the fifth residue of CDR3, TTVVAD, was unique to V_H of MoAb HA58.

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  1  GAG GTC AAG CTG CAG GAG TCA GGA CCT GAG CTG GTG AAG CCT GGG GCT
  1   E   V   K   L   Q   E   S   G   P   E   L   V   K   P   G   A

 49  TCA GTG AAG ATG TCC TGC AAG GCT TCT GGA TCC ACC TTC AGT GAC TAC
 17   S   V   K   M   S   C   K   A   S   G   S   T   F   S   D   Y
                                                            CDR1

 97  TAC ATG AAG TGG GTG AAG CAG AGG CCA TGG AAA GAG CTT GAG TGG ATT
 33   Y   M   K   W   V   K   Q   R   P   W   K   E   L   E   W   I

145  GGA GAT ATT AGT CCT AAC AAT GGT GAT ACT TTC TAC AAC CAG AAG TTC
 49   G   D   I   S   P   N   N   G   D   T   F   Y   N   Q   K   F
         CDR2

193  AAG GGC AAG GCC ACA TTG ACT GTA GAC AAG TCC TCC AGC ACA GCC TAC
 65   K   G   K   A   T   L   T   V   D   K   S   S   S   T   A   Y

241  ATG CAG CTC AAC AGC CTG ACA TCT GAG GAC TCT GCA GTC TAT TAC TGT
 81   M   Q   L   N   S   L   T   S   E   D   S   A   V   Y   Y   C

289  GCA ACT ACG GTA GTA GCT GAC TTT GAC TAC TGG GGC CAA GGC ACC ACT
 97   A   T   T   V   V   A   D   F   D   Y   W   G   Q   G   T   T
             CDR3

337  CTC ACA GTC TCC TCA GCC AAA ACG ACA CCC CCA TCT GTC TAT CCA CTG
113   L   T   V   S   S   A   K   T   T   P   P   S   V   Y   P   L

385  GCC
129   A
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Fig. 1. Nucleotide and deduced amino acid sequences of the heavy chain variable region of MoAb HA58. Lines indicate CDRs 1, 2 and 3.

Transfection of chimeric genes and radioimmunoassay The chimeric expression vector was transfected into heavy chain loss mutant cells HA58-7 by electroporation. After selection in G418 for 2 weeks, 11 clones were tested for antibody production by indirect binding assay. All the clones showed binding activity to Sez627c cells when detected by anti-human IgG Fc antibody as the second antibody, but did not with anti-mouse IgG Fc. A representative result with anti-human IgG Fc antibody is shown in Fig. 2. Only chHA58 showed a high binding activity to Sez627c cells. No significant binding activity was found when using HA58 or BSA.

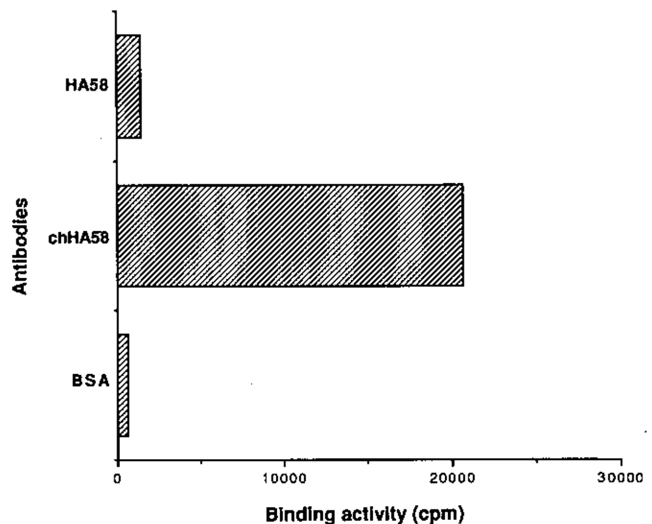

Fig. 2. Reactivity of the supernatant of a chHA58 transfectoma with Sez627c cells. ^{125}I-labeled anti-human IgG-Fc was used as the second antibody. MoAb HA58 and BSA were used as negative controls.

Specificity and affinity of chimeric antibody In order to test the binding activity of chimeric antibody to the antigen, direct binding assay was performed using 9 cultured cells. As shown in Fig. 3, the chimeric MoAb chHA58 clearly reacted with ICAM-1-bearing Sez627c, U937, ATL-16 and RPMI8226 cells. The levels of reactivity of chHA58 to these cell lines were almost identical to those of HA58.

The affinities of mouse MoAb HA58 and chHA58 were similar to each other, i.e., the association constants (K_a) were 2.0×10^8 and 4.1×10^8 M^{-1}, respectively.

Competition assay was done to analyze the spatial relationship among epitopes recognized by mouse MoAb HA58 and chimeric MoAb. Fig. 4 showed that HA58 and chHA58 inhibited the binding of the other ^{125}I-labeled MoAb to Sez627c cells, indicating that the specificity of chHA58 is the same as that of HA58.

ADCC by the chimeric MoAb chHA58 The chimeric MoAb chHA58, possessing the constant region of human origin, was tested for ADCC activity using human effector cells. Normal human peripheral blood mononuclear cells were used as effector cells. In ADCC assay, Sez627c and JRST cells were used as target cells. JRST cells highly expressed ICAM-1 (data not shown). ChHA58 showed little ADCC activity, the level being almost equal to that of HA58 (Fig. 5).

Biodistribution of ^{99m}Tc-labeled chHA58 in nude mice bearing JRST tumors ^{99m}Tc-chHA58 (2 μCi/100 μg) was injected into a tail vein of mice bearing JRST tumors. Organ distributions were measured at 3 and 18 h after injection. As shown in Fig. 6, tumor uptake was 17.5 %ID/g at 18 h after injection, when the blood level was 11.3 %ID/g, and all other organ levels except for the kidney (9.9 %ID/g) were less than 4.5 %ID/g. The tumor-to-blood ratio at that time was 1.55.

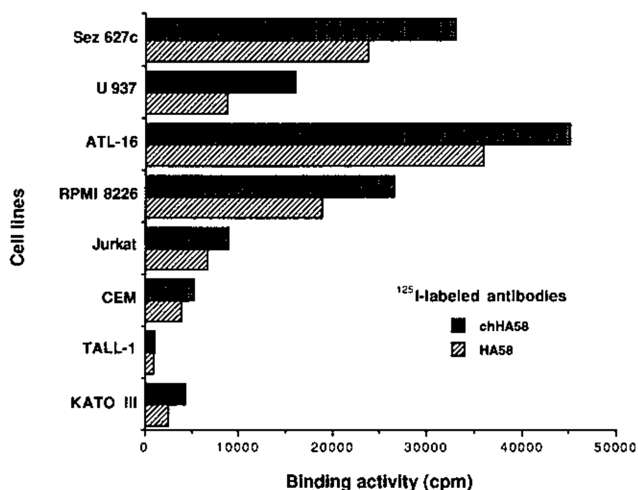

Fig. 3. Comparison of reactivity with cultured cells between MoAbs HA58 and chHA58 by direct binding assay.

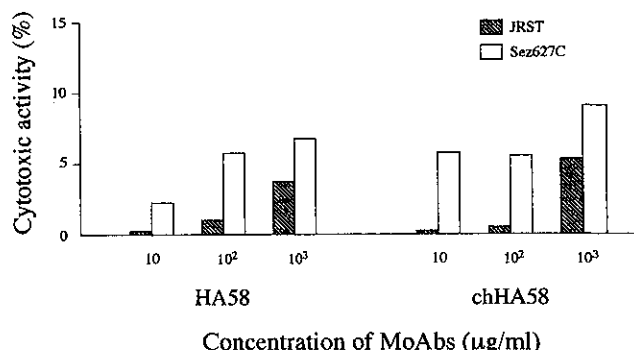

Fig. 5. Comparison of ADCC activity between HA58 and chHA58. MoAbs were used at 10, 100 or 1000 μg/ml.

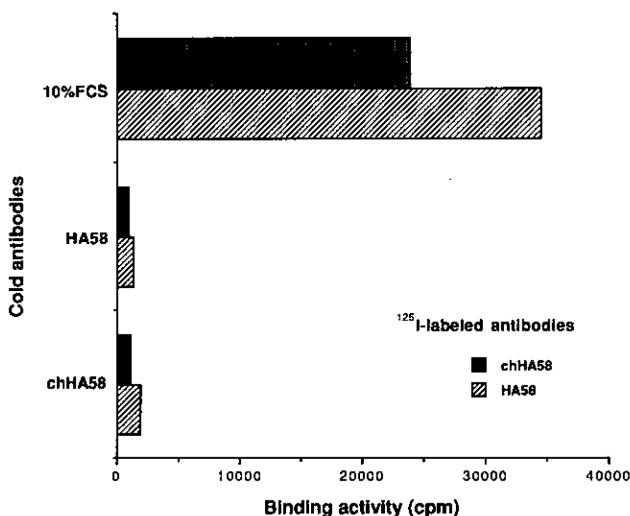

Fig. 4. Competition assay between MoAbs HA58 and chHA58. The reactivity of HA58 with Sez627c was completely inhibited by chHA58 and *vice versa*. FCS was used as a negative control.

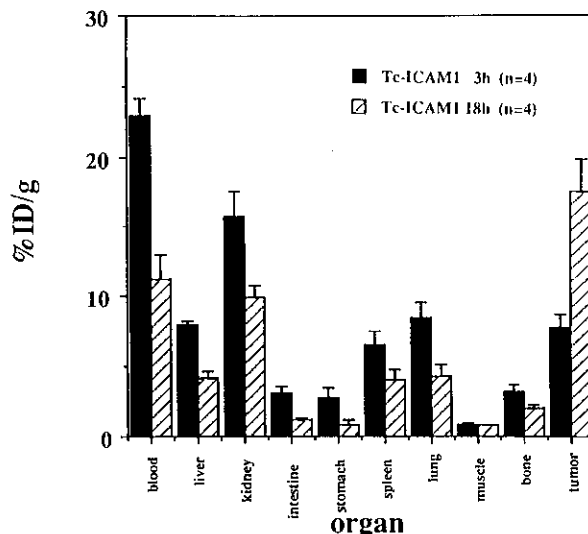

Fig. 6. Biodistribution of Tc-99m labeled MoAb chHA58 in athymic mice bearing JRST tumors.

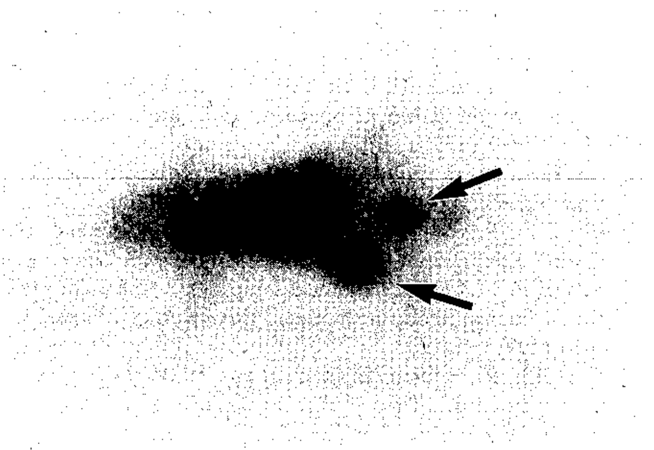

Fig. 7. Gamma scintigraphy of JRST tumor-bearing mouse at 18 h after injection of ^{99m}Tc-labeled chHA58.

Imaging of JRST tumors with ^{99m}Tc Fig. 7 shows the result of gamma scintigraphy of tumor-bearing mice injected intravenously with 200 μCi/10 μg of ^{99m}Tc-labeled chHA58. Images were obtained after 18 h, when the tumors were clearly visible, and confirmed the results of biodistribution studies, showing a higher radioactivity in the tumor and liver of mice. High accumulation was observed in the liver, in spite of the fact that its uptake of labeled antibody was relatively low (Fig. 6). This is probably due to the thickness of the liver.

DISCUSSION

The procedure that we used to obtain mouse/human antibody was originally developed by Xiang *et al.*[12] We have previously reported the preparation of a chimeric antibody against erbB-2 protein by using this procedure.[23] In comparison with the conventional construction of mouse/human chimeric antibodies involving the use of either the genomic V regions,[24] or cDNA V regions,[25] this method has the following advantages[12, 23]: (i) since only the heavy chain V region cDNA fragment was directly cloned from the hybridoma RNA by PCR, the time required is minimal, (ii) the promoter and enhancer of murine immunoglobulin origin were introduced into the expression vector, resulting in a high productivity (2.0 μg/ml hybridoma supernatant) of the chimeric antibody, equal to that of the mouse parental hybridoma, (iii) the binding affinity of the chimeric antibody to the antigen is almost equal to that of the parental mouse MoAb, (iv) the transfectoma stably produces chimeric antibody. The entire light chain of this chimeric antibody is of mouse origin, and it has been shown by previous clinical studies on chimeric antibodies that HAMA can be produced against the variable region as well as against the constant region.[26] In spite of that, HAMA occurred at a later time, in lower titers and less frequently than in patients receiving murine antibodies,[13, 14, 27] suggesting that even the chimeric antibody with mouse C_L and C_H could be less immunogenic *in vivo* than the parental murine antibody.

The acquisition of ADCC activity is one of the advantages of chimeric antibodies against tumor-associated antigens.[23, 28] ChHA58 showed little ADCC effect, probably due to the inhibition of the cell-to-cell interaction through certain ligands, which could be essential to ADCC activity. It was shown that the effector cells mediating ADCC are natural killer (NK) cells and that interleukin-2-activated NK cells have higher ADCC than resting NK cells. We have found that mouse MoAb HA58 partly inhibits the NK cell activity of peripheral blood mononuclear cells (to be published elsewhere). Also, anti-ICAM-1 antibody inhibits the cytolytic activity of various effector cells such as lymphocyte-activated killer cells,[29] cytotoxic T cells,[30] and monocytes.[31, 32] These effects of anti-ICAM-1 antibody would be convenient for radioimmunoimaging of tumors, since there would be little cytotoxicity against the normal cell populations expressing ICAM-1 which surround tumor cells. Indeed, several clinical trials with anti-ICAM-1 MoAb in patients with renal allografts and rheumatoid arthritis have shown safety and nontoxicity in humans.[33, 34]

For radioimmunoimaging, chHA58 was labeled with ^{99m}Tc in this study. ^{99m}Tc has many advantages over radioiodines for clinical use and has been employed mainly in the labeling of MoAbs.[35, 36] An anti-osteosarcoma MoAb OST7 labeled with ^{99m}Tc has been shown to have higher tumor uptake than that labeled with ^{125}I.[37] It has been demonstrated that good tumor uptake requires a reasonably high-affinity MoAb ($K_a > 10^8$ M^{-1}).[38] In this context, the association constant of chHA58 was 4.1×10^8 M^{-1}, suggesting that it is available for tumor imaging. Isobe *et al.* have recently shown that cardiac rejection in mice was clearly detected by radioimmunoscintigraphy using ^{123}I-labeled anti-ICAM-1 MoAb.[39] This suggests that the normal cells around cancer cells, on which high ICAM-1 expression was induced, could be imaged by radiolabeled MoAb, giving rise to the enhancing effect on tumor imaging.

ACKNOWLEDGMENTS

This work was financed by Grants-in-Aid for Cancer Research from the Ministry of Education, Science, Sports and Culture (K.I., Y.H.), and from the Ministry of Health and Welfare (K.I.) of Japan.

(Received October 12, 1995/Accepted December 28, 1995)

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