Anti-CD54 (ICAM-1) Has Antitumor Activity in SCID Mice with Human Myeloma Cells

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ABSTRACT

Recent studies have suggested that ICAM-1 (CD54) is involved in the pathogenesis of human multiple myeloma. A monoclonal antihuman CD54 antibody has been generated by immunizing BALB/c mice with human myeloma cell lines. SCID mice injected with human ARH-77 myeloma cells develop disseminated myeloma which is similar in several respects to multiple myeloma in humans. The mice have monoclonal gammapathy and succumb to hind leg paralysis caused by infiltration of tumor cells into the thoracolumbar vertebrae, resulting in compression of the spinal cord. In the absence of treatment, the mean paralysis time of the SCID/ARH-77 mice is 29 days. When the SCID/ARH-77 mice received four consecutive daily i.v. injections of anti-CD54 mAb commencing 1 day after tumor inoculation, they survived for 150 days, at which time the experiment was terminated. Histopathological analyses indicated that prior to death all control SCID/ARH-77 mice had myeloma cells in the vertebral and skull. At this time, the anti-CD54-treated mice had no evidence of tumor. High levels of human immunoglobulin were detected in the sera of control, but not treated mice. F(ab')2 fragments of the anti-CD54 antibody also had similar, albeit, slightly less antitumor activity in vivo, suggesting that antibody effector function may account for some, but not all the antitumor activity of anti-CD54. In vitro studies indicate that anti-CD54 does not inhibit homotypic adhesion, the binding of myeloma cells to murine bone marrow stromal cells, or cell proliferation. By exclusion, we propose that the CD54-mediated homing of these ARH-77 cells to certain anatomical sites is crucial for their growth in vivo.

INTRODUCTION

During the past 20 years, there has been considerable interest in using mAbs to destroy antigen-bearing tumor cells via ADCC or CML. However, early clinical trials with unmodified mAbs for the treatment of solid tumors have not yielded dramatic successes (1). For the most part, this is due to the fact that patients have advanced, bulky, poorly vascularized tumors. More encouraging results have however, been achieved in lymphoma and leukemia. Recent advances in our understanding of the signaling and regulation of tumor cell growth have provided new rationales for developing therapeutic antitumor mAbs (2). To date, many studies have indicated that mAbs can directly suppress the growth of tumor cells by binding to cell surface molecules, which signal rapid apoptosis or cell cycle arrest (3, 4), directly block the binding of critical growth factor receptors (5), or interfere with the homing of tumor cells to organ sites where the microenvironment is favorable for growth (6). Our previous study (3) indicated that the anti-CD19 antibody, HD37, inhibits B-cell lymphoma cell growth in SCID mice by inducing cell cycle arrest. More recently, Funakoshi et al. (7) observed that an anti-CD54 antibody inhibited homotypic adhesion, the binding of myeloma cells to murine bone marrow stromal cells, or cell proliferation. By exclusion, we propose that the CD54-mediated homing of these ARH-77 cells to certain anatomical sites is crucial for their growth in vivo.

MATERIALS AND METHODS

Production of Monoclonal Anti-CD54 Antibody and Anti-CD54-dgA Immunotoxin. The anti-CD54 antibody, UV3, was produced as described previously (18), using 4-succinimidyloxycarbonyl-a-methyl-a-(2-pyridyldiazo)-thio) toluene. In [3H]leucine protein synthesis inhibition assays, this immunoconjugate inhibited the growth of ARH-77 cells in the vast majority of SCID mice with minimal disease. The IgG is also highly effective in a proportion of mice with more advanced disease. Based on results of in vitro studies, we suggest that the long-term growth of ARH-77 cells in vivo requires CD54-mediated events involving homing of cells to anatomical sites crucial for their growth in vivo.

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2 To whom requests for reprints should be addressed, at Cancer Immunobiology Center, University of Texas Southwestern Medical Center, 6000 Harry Hines Blvd., Dallas, TX 75235.

3 The abbreviations used are: ADCC, antibody-dependent cell-mediated cytotoxicity; CML, complement-mediated cell lysis; MPT, mean paralysis time.
were palpable tumor masses on their legs, mandible, or head. These mice
ous release of 51Cr was measured after incubating 51Cr-labeled cells with
51Cr-labeled cells (1 x 10^5) were incubated with 1/xg/ml of anti-CD54
(treated with medium only. The maximal release of 51Cr was determined as follows:

\[ \text{Experimental } \% \text{ release} = \frac{\text{Experimental specific release}}{\text{Maximal specific release}} \times 100 \]

**Murine Bone Marrow Stromal Cell Culture.** Bone marrow stromal cells
from the vertebrae of SCID mice were grown in Iscove's modified Dulbecco's
medium supplemented with 10% horse serum, hydrocortisone (1 x 10^-2 m;
Sigma, St. Louis, MO), glutamine (100 /m), penicillin (100 units/ml), and
streptomycin (100 /g/ml). The stromal cells were harvested with trypsin-
EDTA buffer (GIBCO, Gaithersburg, MD).

**Cell Adhesion Assay.** 51Cr-labeled myeloma cells were preincubated with
anti-CD54 or UPC-10 (control) for 30 min on ice and then incubated for 4 h
at 37°C with murine stromal cell monolayers derived from the marrow
of SCID mice. The nonadherent cells were removed by washing the adherent cells
3 times. The remaining tumor cells were lysed with 1 n NaOH and radioac-
tivity in the cell lysates was determined using a gamma counter. A peptide,
RGDS (GIBCO), which binds to fibronectin receptors and blocks the ability of
fibronectin to bind to its receptor (21), was used as a positive control. The
adhesion of tumor cells was expressed as the percentage of total added
51Cr-labeled tumor cells.

**Homotypic Adhesion Assay.** ARH-77 cells were resuspended at 1 x 10^6-
5 x 10^6 in RPMI 1640 containing 10% FCS and 100 /g/ml of glutamine and
antibiotics. Cells (200 /g) were plated in wells of 96-well flat-bottom tissue culture plates (Costar, Cambridge, MA). The anti-CD54 or isotype-
matched myeloma protein UPC-10 were then added to a final concentration
of 10-100 /g/ml. Cell aggregation was examined once/day for 3 days using an
inverted phase contrast microscope.

**RESULTS**

**Anti-CD54 Inhibits the Disseminated Growth of Human Myeloma in SCID Mice.** We first determined whether anti-CD54 could
prevent the long-term growth of ARH-77 cells in vivo. SCID mice
were irradiated and then given a single i.v. injection of ARH-77 cells. One
day after tumor inoculation, the mice were treated with 4
consecutive daily i.v. injections of 0.8 /g/g anti-CD54 antibody. As
shown in Table 1 and Fig. 1, anti-CD54 prolonged the survival of
SCID/ARH-77 mice. None of the mice treated with the anti-CD54 mAb
or immunotoxin developed paralysis, but 1 immunotoxin-treated
mouse died from tumor on day 54. All the other anti-CD54- and
anti-CD54-dgA-treated mice survived for 150 days at which time the
experiment was terminated. Histopathological examination indicated
that in both anti-CD54- and anti-CD54-dgA-treated mice, there was 1
animal/group with a small focus of tumor cells in the brain. Other
animals appeared tumor-free. All negative control groups [untreated,
UPC-10 (isotype-matched immunoglobulin), or UPC-10-dgA-treated
mice] developed neurological symptoms such as circling due to the infiltration
and compression of the brain by intracranial tumors. In other cases, there
were palpable tumor masses on their legs, mandible, or head. These mice
developed hind leg paralysis, all mice were bled and sacrificed. Excised
animals appeared tumor-free. All negative control groups [untreated,
UPC-10 (isotype-matched immunoglobulin), or UPC-10-dgA-treated
mice] developed neurological symptoms such as circling due to the infiltration
and compression of the brain by intracranial tumors. In other cases, there
were palpable tumor masses on their legs, mandible, or head. These mice
developed hind leg paralysis, all mice were bled and sacrificed. Excised
organisms including lung, liver, kidney, vertebrae, and head were fixed in
formalin, embedded in paraffin, sectioned, and stained with hematoxylin &
eosin to confirm the presence or absence of tumor by histopathological
examination.

**Serum Immunoglobulin Assay.** The levels of human immunoglobulin in
the sera of SCID/ARH-77 mice were determined by ELISA (16).

**Preparation of F(ab')2 Fragments.** F(ab')2 fragment was prepared by
pepsin digestion and purified by HPLC gel filtration (18). The purity of the
fragments was determined by SDS-PAGE. The binding activity of the F(ab')2
fragments was determined by flow cytometry using ARH-77 cells and a
FITC-labeled monoclonal rat anti-mouse / light-chain antibody (RAMK;
Tago, Burlingame, CA).

**In Vivo Therapy with F(ab')2 Fragments of Anti-CD54.** Four or 8
/ g/day of F(ab')2 fragments were administered to SCID mice for 4 days
commencing 1 day after tumor inoculation.

**Cell Proliferation Assay.** The effect of the anti-CD54 antibody on the
proliferation of human myeloma cell line ARH-77 was determined using a
[3H]thymidine incorporation assay (20). The ARH-77 cells were distributed
into wells of 96-well culture plates at 5 x 10^4 cells/ml at 200 /¿I/well and
incubated with various amounts of anti-CD54 or control myeloma protein
UPC-10 for 24 h. After that time, the cells were pulsed for 24 h with 1
1 Ci/well of [3H]thymidine (Amersham) and then harvested onto glass filters
using an automated cell harvester (Skatron, Sterling, VA). The incorporated
radioactivity was measured using a liquid scintillation counter. The results
were expressed as a percentage of the [3H]thymidine incorporated by cells
treated with medium only.

**Complement-mediated Lysis.** Lysis was determined using a 51Cr release
assay with rabbit rabbit complement. ARH-77 cells were labeled with 51Cr
(75 /Ci/10^6 cells, sodium chromate, Amersham Corp., Arlington Heights,
IL) at 37°C for 4 h. For the cells were then washed 3 times with RPMI 1640.
51Cr-labeled cells (1 x 10^5) were incubated with 1 /g/ml of anti-CD54 antibody
(or UPC-10) on ice for 30 min. The unbound antibody was removed
by washing the cells three times with RPMI 1640. The cells were then
distributed into 96-well plates and incubated with serial dilutions of
rabbit rabbit complement (Pel-Freeze, Rogers, AR) at 37°C for 2 h. After
incubation, the plates were centrifuged at 400 g for 5 min. Supernatants
from each well (50 /g) were harvested and 51Cr was measured. Spontane-
ous release of 51Cr was measured after incubating 51Cr-labeled cells with
medium alone. The maximal release of 51Cr was determined after incuba-
tion of 51Cr-ARH-77 cells with 5% Triton X-100. Specific CML was
determined as follows:

\[ \text{Experimental } \% \text{ release} = \frac{\text{Experimental specific release}}{\text{Maximal specific release}} \times 100 \]
with antibody alone or with immunotoxin. Groups of mice were treated from days 1–4 with the same amounts (0.8 µg/g) of anti-CD54, UPC-10-dgA, anti-CD54-dgA or an equal volume of PBS. When the PBS-treated mice exhibited hind leg paralysis, all mice were bled and sacrificed. The presence or absence of tumor in the head, vertebrae, liver, kidney, and lung was evaluated by histopathology (Fig. 2, Table 2). The animals in the control groups (PBS or UPC-10-dgA) had widespread tumor. As observed previously in this animal model, the tumor has a propensity to proliferate in narrow cavities in vertebral bodies: the tumor cells destroy vertebrae and invade the spinal canal and perivertebral tissues. Tumor cells were also found in the lung, kidney, nasal cavities, meninges, and bones of the head. In contrast, as determined by histological studies, no tumor cells were found in any of the anti-CD54 or anti-CD54-dgA-treated animals. No overt toxicity was detected in the anti-CD54-treated mice, but the animals treated with anti-CD54-dgA had scattered mild skeletal muscle myodegeneration due to the toxicity of dgA (data not shown) (22).

**Human Immunoglobulin in the Sera of SCID/ARH-77 Mice.** Serum concentrations of human immunoglobulin in treated mice were measured by ELISA. High levels of human immunoglobulin (44–48 µg/ml) were detected in the control animals (Table 3); the anti-CD54- or anti-CD54-dgA-treated animals had low levels (0.02–0.60 µg/ml).

**Anti-CD54 Inhibits the Growth of Advanced Tumor in SCID Mice.** We also determined whether the same amounts of anti-CD54 antibody (0.8 µg/g/day) would demonstrate antitumor activity in SCID mice with more advanced tumors. Antibody treatment was, therefore, initiated 8 or 15 days after tumor inoculation. As shown in Table 4, anti-CD54 prolonged the survival of SCID/ARH-77 mice when the treatment was commenced on day 8; only 1 of 5 mice had vertebral tumor, although all mice eventually developed large focal tumors in other organs such as femur, mandible, or brain. The suppression of tumor growth was less effective if the treatment was started on day 15, and 60% of mice in this group developed hind leg paralysis with a MPT of 47.3 days. One mouse died without tumor on day 123, and 1 remaining mouse was sacrificed at 150 days with no evidence of tumor.

We further determined whether increasing the dose of the antibody would increase its in vivo antitumor activity in mice with advanced tumors. To this end, 4 µg/g/day × 4 days of anti-CD54 or UPC-10 (control) were administered commencing on days 8 or 15. As shown in Table 5, a 5-fold increase in the dose of antibody markedly enhanced its antitumor activity in mice with advanced tumors; 80% of the anti-CD54-treated mice survived for more than 124 days. Nevertheless, survival was prolonged, tumors still grew in some anatomical sites. It is therefore possible that anti-CD54 prolongs survival by preventing infiltration of the tumor cells into the vertebral column and hence, delays paralysis. All untreated or UPC-10-treated mice developed tumor with a MPT of 35–37 days. These results indicate that anti-CD54 treatment is also efficacious in mice with established tumors. Future studies will be aimed at testing even higher doses in an effort to further improve antitumor activity.

**The Inhibitory Effect of F(ab')2 Fragment of Anti-CD54 Antibody.** The anti-CD54 antibody could exert antitumor activity by Fc-mediated effector functions (e.g., ADCC or CML), by negative signaling, or both. Therefore, we next determined whether F(ab')2 fragments of anti-CD54 had antitumor activity in vivo. The F(ab')2 fragments of the antibody were prepared by enzymatic digestion and then purified by HPLC. Binding activity on ARH-77 tumor cells was determined by flow cytometry (Fig. 3). We also examined the ability of the F(ab')2 fragments to elicit complement-mediated lysis. As shown in Table 6, the intact antibody mediated lysis while the F(ab')2 fragments did not.

As shown in Table 7, when 4 µg/g/day of F(ab')2 fragments were administered, only 1 of 5 mice developed paralysis on day 59. Three mice had tumors on days 52, 55, and 102, which were located in perivertebral connective tissue and muscle, the left scapula and the left femur, respectively. One mouse survived for 150 days and was tumor free (Fig. 2). As compared to control mice (all mice were paralyzed with a MPT of 29.6 days), the F(ab')2 fragment induced prolongation of survival. Since F(ab')2 fragments are cleared 10-fold more rapidly than IgG in vivo (3), as expected from the suboptimal dose of F(ab')2 fragment used in this experiment (5-fold higher than intact IgG) complete inhibition of tumor growth was not achieved. Therefore, we next used 10-fold more F(ab')2 than IgG (8 µg/g/day) to treat the animals. As shown in Table 7, 50% of the mice survived for more than 150 days without tumor. One mouse died from infection on day 123, and another mouse had hind leg paralysis on day 50. Three mice had a tumor on day 78 and 91, and the tumors were located in the left front leg, right femur, and perivertebral tissues, respectively. In contrast, all untreated mice and 80% of mice treated with control mIgG F(ab')2.
Fig. 2. The antitumor activity of anti-CD54 in SCID mice with disseminated human myeloma (ARH-77). A, vertebral marrow from PBS-treated SCID/ARH-77 mouse sacrificed on day 29 after tumor inoculation. Myeloma cells have replaced the hematopoietic cells. B, vertebral marrow from a UPC-10-dgA-treated SCID/ARH-77 mouse sacrificed on day 29. Myeloma cells have replaced the hematopoietic cells. C, vertebral marrow from an anti-CD54 treated mouse sacrificed on day 29. The hematopoietic elements are normal. D, E, F, vertebral marrow from anti-CD54-dgA, IgG anti-CD54, and F(ab')2 anti-CD54-treated mice, respectively, sacrificed on day 150. The hematopoietic elements are normal. × 400.

developed tumors with MPTs of 48 and 38 days. These results demonstrate that higher doses of F(ab')2 anti-CD54, which offset their shorter half-life, have antitumor activity in vivo, albeit, less activity than IgG anti-CD54. We conclude that the Fc portion of the mAb plays a partial role in its antitumor activity.

Anti-CD54 Does Not Interfere with Homotypic Adhesion or the Adhesion of ARH-77 Myeloma Cells to Murine Stromal Cells in Vitro. One possible explanation for the inhibitory activity of anti-CD54 is that it prevents the tumor cells from interacting with each other or with bone marrow stromal cells. The latter appeared unlikely since human CD54 does not interact with murine CD11a/CD18 (23). Nevertheless, a cell adhesion assay was performed to determine whether the anti-CD54 affected the binding of ARH-77 cells to murine marrow stromal cells or to each other. The results were negative (Table 8; data not shown). In contrast, the peptide, RGDS, which binds to fibronectin receptors and blocks the interaction of fibronectin with its receptor (21), significantly inhibited the binding of ARH-77 cells to stromal cells. This experiment suggests that the
antitumor effect of anti-CD54 cannot be attributed to its ability to interfere with the attachment of tumor cells to narrow stromal cells or to inhibit homotypic adhesion.

Anti-CD54 Does Not Inhibit the Short-Term Proliferation of ARH-77 Cells in Vitro. Since anti-CD54 inhibits the growth of ARH-77 cells in SCID mice, we next determined whether anti-CD54 (like some other mAbs) would inhibit [3H]thymidine incorporation in ARH-77 cells in vitro. Tumor cells were incubated with Abs for 24 h and then pulsed with [3H]thymidine for 24 h. As shown in Table 9, even at 1 mg/ml, no inhibitory effects were detected when the antibody was added to cultures of ARH-77 cells for 48 h.

**Table 2:** Antitumor effect of anti-CD54 antibody in SCID mice with ARH-77 myeloma using histopathological analysis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Site of tumor growth</th>
<th>No. of mice with tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>Vertebral column</td>
<td>5/5</td>
</tr>
<tr>
<td>UPC-10-dgA (control)</td>
<td>5/5</td>
<td>4/5</td>
</tr>
<tr>
<td>Anti-CD54</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Anti-CD54-dgA</td>
<td>0/5</td>
<td>0/5</td>
</tr>
</tbody>
</table>

*a* The mice were sacrificed 29 days after tumor inoculation.

**Table 3:** Human immunoglobulin in the sera of SCID mice with ARH-77 myeloma

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Human immunoglobulin (mg/ml) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>47.74 ± 9.63</td>
</tr>
<tr>
<td>UPC-10-dgA</td>
<td>44.59 ± 19.20</td>
</tr>
<tr>
<td>Anti-CD54</td>
<td>0.58 ± 0.28</td>
</tr>
<tr>
<td>Anti-CD54-dgA</td>
<td>0.02 ± 0.02</td>
</tr>
</tbody>
</table>

*a* Five mice/group.

**DISCUSSION**

In this study, we have shown that an anti-CD54 monoclonal antibody has significant antitumor activity in SCID mice with disseminated human ARH-77 multiple myeloma. This antitumor activity is equal to that induced by the corresponding immunotoxin. When administered early in the course of the disease, anti-CD54 almost completely prevented tumor growth. When given later in the course of the disease, the antibody was less effective, but still induced complete remissions in about 20% of the mice. Importantly, when 5-fold-more antibody was administered, it prolonged survival in 80% of mice with more advanced diseases. It appears that this may be due to the ability of the antibody to prevent the tumor cells from infiltrating the spinal canal and causing paralysis. The precise mechanisms by which the anti-CD54 antibody exerts its antitumor activity are not yet well understood. We have excluded the possibility that the effect is due entirely to Fc-mediated effector functions since F(ab')2 fragments were also highly effective when larger amounts were administered to offset their shorter half-life. However, the Fc does play some role since the IgG is more effective than the F(ab')2 at doses adjusted for differences in their t½ values. Thus, ADCC or C1-mediated lysis may give the IgG molecules improved antitumor activity.

We have ruled out the possibility that antitumor activity is due to short-term antiproliferative effects of the antibody since anti-CD54 has no effect on [3H]thymidine incorporation by ARH-77 cells in vitro. This observation is consistent with the results of a recent report by Kawano et al. (24) who found that anti-CD54 inhibited neither spontaneous proliferation nor IL-6-mediated proliferation of myeloma cells in short-term culture. Because human CD54 does not interact with murine CD11a/CD18 (22) and because anti-CD54 does not react with murine CD54 (data not shown) it is unlikely that the anti-CD54 antibody exerts its antitumor activity by blocking the interaction between human CD54 and murine CD11a/CD18. Our data support this interpretation.

We also failed to demonstrate that anti-CD54 inhibits homotypic adhesion of tumor cells in vitro. Our observations differ from those of Kawano et al. (24) who reported that CD54/CD11a/CD18 interactions are involved in the homotypic adhesion of myeloma cells in vitro. It has also been reported that several cytokines and growth factors such as IL-6 (25, 26), IL-3 (27), IL-1 (28), and granulocyte-macrophage colony-stimulating factor (29) augment the proliferation of myeloma cells and that human myeloma cells can secrete autocrine cytokines (30–32). It is therefore possible that homotypic adhesion of just a few myeloma cells induces them to secrete autocrine growth factors which promote their growth in vivo. In this regard, anti-CD54 may prevent the secretion of autocrine growth factors necessary for in vivo growth, but it may not be possible to readily document this effect using "high density" in vitro cultures.

By exclusion, we favor the possibility that anti-CD54 prevents events involved in the homing of tumor cells to certain organs sites and/or signaling in vivo. In this regard, Ahsmann et al. (12) demonstrated that expression of CD11a/CD18 on plasma cells correlates well with tumor labeling index. In contrast to normal plasma cells, which do not express CD11a/CD18, malignant myeloma cells (including ARH-77 cells) do (21, 30).

Experiments are currently under way to determine whether the down-regulation of CD54 in myeloma cells (using CD54 antisense...
oligonucleotides) reduces their tumorigenic potential in SCID mice. This may provide further insights into the role of this adhesion molecule in the pathogenesis of myeloma.

In considering the therapeutic use of anti-CD54 in humans, it is important to determine whether the anti-CD54 antibody will damage normal tissues that express CD54. Extensive immunohistochemical studies (17, 33, 34) have shown that the cellular distribution of CD54 on normal tissues is restricted. CD54 is usually expressed on endothelium and on reticular dendritic cells in lymph nodes. Cosimi et al. (35) have demonstrated that the administration of anti-CD54 to cynomologous monkeys with renal allografts prolongs graft survival and does not damage normal tissues. More recently, several clinical trials with anti-CD54 in patients with renal allografts (36) and rheumatoid arthritis (37) have demonstrated that anti-CD54 is safe and nontoxic in humans.

In summary, the present study demonstrates that this anti-CD54 antibody prevents the growth of ARH-77 tumors in SCID mice perhaps by altering the homing and growth of cells in certain anatomical sites. The role of this adhesion molecules in myeloma growth has important implications for the pathogenesis of myeloma as well as the therapeutic use of anti-CD54. It remains to be determined whether anti-CD54 will inhibit the growth of myeloma cells in humans in the same manner as it inhibits the growth of ARH-77 cells in SCID mice.

### Table 6 Antibody-dependent complement-mediated cytotoxicity

<table>
<thead>
<tr>
<th>Antibody treatment</th>
<th>Complement dilution (% Cr release ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1:200</td>
</tr>
<tr>
<td>None</td>
<td>1.6 ± 1.6</td>
</tr>
<tr>
<td>IgG-UPC-10 (control)</td>
<td>2.5 ± 2.9</td>
</tr>
<tr>
<td>IgG-anti-CD54</td>
<td>3.4 ± 1.4</td>
</tr>
<tr>
<td>F(ab')2-anti-CD54</td>
<td>1.0 ± 1.7</td>
</tr>
</tbody>
</table>

* a Antibody concentration = 1 μg/ml.
  
* b Complement dilution = 1 μg/ml.
  
* c Experimental % Cr release – Spontaneous % Cr release
  
* d Maximal % Cr release – Spontaneous % Cr release × 100%

### Table 7 Antitumor activity of F(ab')2 fragments of anti-CD54 in SCID/ARH-77 mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Amount of antibody administered (μg/g/day × 4)</th>
<th>Tumor take rate</th>
<th>No. of mice with paralysis</th>
<th>MPT (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td></td>
<td>5/5</td>
<td>5/5</td>
<td>29.6</td>
</tr>
<tr>
<td>F(ab')2-anti-CD54</td>
<td>4.0</td>
<td>4/5</td>
<td>1/5</td>
<td>50.0</td>
</tr>
</tbody>
</table>

* a Positive mice/total mice.

* b One mouse had hind leg paralysis on day 59. Three mice had tumors on days 52, 55, and 102, and the tumors were located on perivertebral, left scapula, and left femur, respectively. The remaining mouse survived >150 days.

* c Two mice died from tumor on days 40 and 43, respectively.

* d One mouse had a tumor in its left femur on day 107. Another two mice survived >150 days.

* e One mouse had hind leg paralysis on day 50. One mouse had a tumor in its left front leg on day 78. On day 91, another two mice had tumors in their pelvic cavity and right femurs, respectively. One mouse died from infection on day 123. Five remaining mice survived >150 days.

* f This peptide blocks the binding site of the fibronectin receptor and prevents cell-cell adhesion.

* g Positive mice/total mice.

* h One mouse had hind leg paralysis on day 50. One mouse had a tumor in its left front leg on day 78. On day 91, another two mice had tumors in their pelvic cavity and right femurs, respectively. One mouse died from infection on day 123. Five remaining mice survived >150 days.

* i One mouse had a tumor in its left femur on day 107. Another two mice survived >150 days.

* j One mouse had hind leg parallels on day 50. One mouse had a tumor in its left front leg on day 78. On day 91, another two mice had tumors in the pelvic cavity and right femurs, respectively. One mouse died from infection on day 123. Five remaining mice survived >150 days.
ACKNOWLEDGMENTS

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