Mouse-Human Chimeric Antibody against the Multidrug Transporter P-Glycoprotein

Hirofumi Hamada, Keiji Miura, Keiko Ariyoshi, Yuji Heike, Shigeo Sato, Koh-zoh Kameyama, Yoshikazu Kurosawa, and Takashi Tsuruo


ABSTRACT

In an effort to devise an effective treatment for human drug-resistant cancers, we have generated a monoclonal antibody, MRK16, reactive to the multidrug transporter P-glycoprotein. The monoclonal antibody inhibited the growth of human drug-resistant tumor cells in a xenograft model, suggesting its potential usefulness in the immunotherapy of drug-resistant cancers. In this study, we have developed a recombinant chimeric antibody in which the antigen-recognizing variable regions of MRK16 are joined with the constant regions of human antibodies. When human effector cells were used, the chimeric antibody, MH162, was more effective in killing drug-resistant tumor cells than the all-mouse monoclonal MRK16. The chimeric antibody against the multidrug transporter P-glycoprotein will be a useful agent in immunotherapy of human drug-resistant cancers.

INTRODUCTION

Resistance of tumors to a variety of chemotherapeutic agents presents a major problem in cancer treatment. Resistance to such agents as doxorubicin (Adriamycin), Vinca alkaloids, and actinomycin D can be acquired by tumor cells after treatment with a single drug (1, 2). The gene responsible for multidrug resistance, termed mdr1, encodes a membrane glycoprotein (P-glycoprotein) that acts as a pump to transport various cytotoxic drugs out of the cell (3). The P-glycoprotein has been shown to bind anticancer drugs (4, 5) and to be an ATPase (6, 7) located at the plasma membrane of resistant cells (8, 9). Transfection of cloned mdr sequences confers multidrug resistance on sensitive cells (10–12).

The amount of P-glycoprotein expression has been measured in tumor samples and was found to be elevated in intrinsically drug-resistant cancers of the colon, kidney, and adrenal as well as in some tumors that acquired drug resistance after chemotherapy (13–15). Because P-glycoprotein appears to be involved in both acquired multidrug resistance and intrinsic drug resistance in human cancer, the selective killing of tumor cells expressing P-glycoprotein could be very important for cancer therapy.

In an effort to devise an effective treatment for human drug-resistant cancers, we developed monoclonal antibodies reactive to the multidrug transporter P-glycoprotein (16). The monoclonal antibodies, given i.v., effectively prevented tumor development in athymic mice inoculated s.c. with drug-resistant human ovarian cancer cells (17). Treatment with one of the monoclonal antibodies, MRK16, induced rapid regression of established s.c. tumors and cures of some animals. These monoclonal antibodies may have potential as treatment tools against multidrug-resistant human tumors that possess P-glycoprotein (17). However, the mouse antibodies, as foreign proteins, may evoke counteracting immune reactions that could destroy their effectiveness and may also cause allergic reactions in the patients (18, 19). In this study, we constructed recombinant chimeric antibodies in which the antigen-recognizing variable regions of MRK16 are joined with the constant regions of human antibodies (20, 21). When human effector cells were used, the chimeric antibody was much more effective in killing drug-resistant tumor cells than the all-mouse MRK16, as determined by antibody-dependent cell-mediated cytolysis.

MATERIALS AND METHODS

Vectors, Clones, Probes, and Cells. λ phage λgt10 (22) and λ EMBL3 (23) were used as EcoRI and BamHI vectors, respectively. pSV2HG1gpt and pSV2HC, neo2 were constructed as described previously (24). Mouse J, gene-containing fragment (J, probe) was isolated from clone Ig146 (25). Mouse J, probe was isolated from MEP203 (26). Mouse myeloma SP2/0 was obtained from ATCC (Rockville, MD). The hybridoma that produced the monoclonal antibody MRK16 was generated and maintained as described previously (16). Human drug-resistant cell lines (K562/ADM and 2780(10)) and their parent drug-sensitive cell lines (K562 and A2780, respectively) were maintained as described previously (27). Anti-phosphorylcholine mouse-human chimeric antibody was prepared and purified as reported previously (24).

Cloning of Size-fractionated DNA. Active V gene-containing fragments were identified as rearranged bands in Southern hybridization (28) of BamHI- and EcoRI-digested genomic DNAs with J, and J, probes, respectively. DNAs were eluted from the relevant regions, separated on agarose gel, ligated with λEMBL3 and λgt10 arms, and packaged into λ phage (18, 19). Plaque hybridization was carried out according to the method of Benton and Davis (29).

DNA Transfection of Mouse SP2/0 Myeloma Cells. Two hundred μg each of plasmids pSV2-VH16-HG1gpt and pSV2-V,-16HC,-neo (see Fig. 1) were cotransfected into 107 mouse SP2/0 cells (CRL1581; ATCC) by electroporation (30, 31). Transformants were selected in RPMI 1640 medium supplemented with 10% fetal bovine serum and 0.8 mg/ml of G418 (GIBCO, Grand Island, NY). Human antibody in the growth medium was detected by enzyme-linked immunosorbent assay (16, 27).

Isolation of Chimeric Antibody. Antibody-producing cells were grown in RPMI 1640 medium supplemented with 1.0% fetal bovine serum, which had been preclarified of Protein A-binding bovine immunoglobul in affinity chromatography using Protein A-Sepharose CL-4B (Pharmacia, Uppsala, Sweden) (32). Purification of the antibody was carried out as described previously (33), using Protein A-Sepharose CL-4B affinity chromatography.

Cell-binding Radioimmunoassay. Mouse-human chimeric antibody was labeled with [14C]leucine as described (34). Briefly, tens of millions of the myeloma cells producing MH162 were labeled by metabolic incorporation of 50 μCi of i-[14C]leucine (New England Nuclear; 344.0 mCi/mmole) per 30 ml of leucine-free RPMI 1640 medium

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2 To whom requests for reprints should be addressed.

3 The abbreviations used are: neo, Tn5 neomycin resistance gene; Eco-gpt, Escherichia coli xanthine-guanine phosphoribosyl transferase gene; SP2/0, SP2/0-Ag14, ATCC, American Type Culture Collection; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; ADCC, antibody-dependent cell-mediated cytolysis.

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cells lysed with 1% Triton X-100).

The percentage of specific cytolysis was calculated from 51Cr counted in a gamma counter. Determination was carried out in triplicate.

RESULTS

Construction of the Chimeric Heavy Chain Gene. The rearranged variable region (V-D-J) gene of mouse heavy chain was cloned from MRK16-producing hybridoma cells as a 3.0-kilobase EcoRI fragment using the λgt10 library. The variable region gene had rearranged to the J3 segment. The mouse variable region gene was used as an EcoRI fragment for constructing a chimeric heavy-chain gene (Fig. 1A). The mouse variable region gene was joined to a 5' site of the human constant region in the same transcriptional direction, resulting in construction of pSV2-VH16-HG1gpt (Fig. 1A).

Construction of the Chimeric Light Chain Gene. The light chain variable region gene was cloned from genomic DNA of MRK16-producing hybridoma, as a 11-kilobase BamHI fragment using the λEMBL3 library. The variable region gene had rearranged to the J1 segment. The multicloning site from pBluescript SK M13+ (Stratagene, La Jolla, CA) was induced into the HindIII site of pSV2HC, neo (Fig. 1B). Then the mouse variable region gene was trimmed to a 7-kibase BamHI-Xbal fragment and was subcloned into the BamHI-Xbal site of pBluescript SK M13+. The resultant 7-kilobase fragment of the mouse variable region was cut out by NotI/SalI digestion and was cloned into the multicloning site of the pSV2HC, neo. Thus, the mouse light-chain variable region gene was joined to a 6' site of the human constant region in the same transcriptional direction, resulting in construction of pSV2-VH16-HC, neo (Fig. 1B).
A million cells were incubated at 37°C for 60 min with 5000 dpm of [3H]-leucine-labeled MH162 (1 μg of antibody/ml) in 200 μl of PBS supplemented with 5% fetal bovine serum. The cells were then washed with PBS twice, and cell-bound radioactivity was counted in a liquid scintillation counter. Values represent duplicate determinations.

Table 1

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Cell-bound radioactivity (dpm)</th>
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<tbody>
<tr>
<td>K562</td>
<td>33 ± 7*</td>
</tr>
<tr>
<td>K562/ADM</td>
<td>1335 ± 61</td>
</tr>
<tr>
<td>A2780</td>
<td>39 ± 18</td>
</tr>
<tr>
<td>2780AD</td>
<td>923 ± 12</td>
</tr>
</tbody>
</table>

Fig. 3. SDS-PAGE analysis of mouse-human chimeric antibody MH162. Five-tenths μg each of monoclonal antibodies MRK16 (Lane 1) and MH162 (Lane 2) were subjected to SDS-PAGE analysis either with (A) or without (B) mercaptoethanol treatment. Molecular weight markers were obtained from Amersham, Tokyo, Japan.

ADM and 2780AD bound the chimeric antibody to approximately the same extent as the original mouse MRK16 (16, 37). On the other hand, parent drug-sensitive lines K562 and A2780 did not bind either. Thus, this MH162 has the same binding specificities as MRK16, which was previously reported (16, 37).

SDS-PAGE Analysis of the Chimeric Antibody. The chimeric antibody MH162 was purified to apparent homogeneity by single-step affinity chromatography using Protein A-Sepharose (Fig. 3).

Antibody-dependent Cell-mediated Cytotoxicity by the Chimeric Antibody. The chimeric MH162 was used in ADCC assays with human mononuclear cells as effectors. Fig. 4 shows the findings of a representative experiment in which 2780AD cells were exposed to 1 μg/ml of antibody and varying doses of human effector cells. The chimeric MH162 gave significant cytotoxicity even at a 2:1 effector:target cell ratio, while the mouse MRK16 or the control anti-phosphorylcholine mouse-human chimeric monoclonal antibody showed no significant level of specific ADCC activity. Cells from the parent A2780 line were not lysed by the chimeric MH162 or by mouse MRK16 (data not shown). Independent experiments using effector cells from four different donors showed essentially the same results as shown in Fig. 4.

Fig. 4. ADCC with MH162 or MRK16 for 2780AD cells. ADCC was carried out as described in “Materials and Methods” using 1 μg/ml of MH162 (O), 1 μg/ml of MRK16 (C), 1 μg/ml of anti-phosphorylcholine mouse-human chimeric antibody (A), or without monoclonal antibody (A). Points, mean of triplicate determinations; bars, SD. E/T ratio, effector/target ratio.

DISCUSSION

It has long been hoped that monoclonal antibodies directed against tumor cell surfaces could be used in cancer therapy (38). In some cases, monoclonal antibodies alone can inhibit the growth of tumor cells (17, 39–41), whereas in other systems, inhibition of tumor growth can be achieved by complexing the antibody with various toxic substances (42–44). Monoclonal antibodies can be used also in vitro to eradicate residual malignant cells (45). Although such treatments have encountered numerous difficulties (38), some recent successes have been reported (46), and immunotherapy will be of great clinical value in the future.

A major limitation in the clinical use of murine-derived monoclonal antibodies is the immune response elicited against foreign protein, which may render the antibody ineffective and may also harm the patient (18, 19, 38). Furthermore, mouse monoclonal antibodies may interact less efficiently with human effector cells to mediate tumor destruction. Although treatment with human monoclonals is under investigation, human hybridoma cell lines are largely unavailable and, where they do exist, are usually unstable and produce low amounts of immunoglobulin (47). One approach to circumvent the antigenicity of a murine monoclonal antibody in humans is to construct murine variable/human constant chimeric immunoglobulins. Because most immunoglobulin antigenicity resides in the constant domain, creation of murine variable/human constant chimeric immunoglobulin should result in an antibody that has the specificity of a murine monoclonal, yet fails to elicit a human immune response (20, 48, 49). Furthermore, such chimeric proteins may interact more effectively with the human cellular immune system by virtue of their human constant domain and thus provide more beneficial therapy than would the corresponding murine antibody (50). Mouse-human chimeric antibodies were shown to retain their ability to react with hapten antigens (51–53) as well as some carcinoma-associated antigens (54–57). Some trials in cancer immunotherapy using these chimeric antibodies are now in progress (58).

In the current studies, we have developed a mouse-human chimeric antibody against the multidrug transporter P-glycoprotein. Transfection of expression vectors containing chimeric mouse variable/human constant immunoglobulin genes into mouse myeloma cells resulted in the production of functional chimeric IgG with the similar affinity and binding specificity as the original hybridoma antibody. This chimeric antibody should
be much less immunogenic than the mouse antibody. In addition, the human constant regions of the chimeric antibody may more effectively carry out the human effector functions. In fact, when human effector cells were used in the antibody-dependent cell-mediated cytolysis assay, the chimeric antibody MH162 was significantly more effective in killing drug-resistant tumor cells than the all-mouse monoclonal antibody MRK16 (Fig. 4). The cytotoxicity data should be discussed in relation to the isotype of the chimeric antibody. Stepiewski et al. (49) have compared the biological activity of human-mouse IgG1, IgG2, IgG3, and IgG4 chimeric monoclonal antibodies with anticolorectal cancer specificity. They have found differences in the lytic reactivities of chimeric antibodies of various isotypes and concluded that chimeric IgG1 antibody is superior in its antitumor activity. In our current studies, we have at first developed and characterized a chimeric antibody of the IgG1 isotype. It remains to be determined whether our chimeric IgG1 antibody is superior to those of other isotypes (IgG2, IgG3, and IgG4). It also remains to be studied whether or not the chimeric antibody inhibits growth of xenotransplants in nude mice, similar to the inhibition obtained with the murine antibody.

The expression of the P-glycoprotein has now been measured in several human cancers (13–15), and many cancers have been found to contain elevated amounts of P-glycoprotein (13–15). However, a problem might arise from the fact that the P-glycoprotein is constitutively expressed in some normal human organs and tissues including adrenal, kidney, liver, colon (14, 59, 60), and capillary endothelial cells in the brain (61, 62). Presumably, the function of P-glycoprotein in these tissues is to transport normal metabolites or cytotoxic compounds into the blood, urine, bile, or lumen of the colon (61–63). Since MRK16 and MH162 are not cross-reactive to multidrug-resistant mouse tumors (16, 17), it would be difficult to detect undesirable side effects with these antibodies in the murine model systems (17). In humans, undesirable side effects of the anti-P-glycoprotein antibody might occur with respect to the normal function of the organs mentioned above (14, 58–62). Further toxicological studies should be carried out before clinical applications of the chimeric antibody.

It is clear that the true role of this chimeric antibody in the therapy of malignant disease remains to be established. The antibody will certainly be used in conjunction with other effective modalities of treatment rather than as a substitute for them. The important limitation is the finite capacity of the effector cell system that eliminates antibody-coated cells in vivo. Perhaps methods will be found to enhance or augment this system. Alternatively, it may be possible to make the antibody more toxic by arming it with radioactive or toxic substances (42–44). Solid tumors may not be as susceptible to the therapeutic effects of antibodies as are the leukemias and lymphomas, which, as single cells, are exposed to the vascular and reticuloendothelial effector systems. Ultimately, whether the antibody is used for leukemias or for solid tumors, it seems likely that the antibody will have maximum effect when the number of target cells is low. It is for this reason that clinical trials of antibody therapy will eventually need to be done as properly randomized, controlled studies in patients who are in remission but who are at high risk for eventual relapse.

While some problems still exist in the use of MH162 in immunotherapy (17, 58), the chimeric antibody against the multidrug transporter P-glycoprotein might become a useful agent in the treatment of human drug-resistant cancers, and further investigations are warranted.

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