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We have generated a panel of 100 anti-Her-2 MAbs and have characterized them with regard to isotype, epitope recognition and ability to signal apoptosis in Her-2-overexpressing prostate cancer cell lines. Twelve of these MAbs, recognizing nine different epitopes on the Her-2 molecule, negatively signal Her-2-overexpressing breast tumor cells. In parallel work which we are carrying out using MAbs against human lymphoma cells, we have observed that chemically prepared tumor-reactive MAb homodimers (IgG-IgG) of MAbs induce significantly more growth arrest and death than their monomeric (IgG) counterparts (11), probably because of hypercrosslinking (12). In our original application, we proposed evaluating the antitumor activity of our best 12 anti-Her-2 dimers on prostate carcinomas. In the first six months of the project, we prepared IgG homodimers of representative MAbs recognizing the nine different epitopes on the Her-2 molecule. These homodimers were evaluated for their ability to induce apoptosis in Her-2-overexpressing human prostate cancer cell lines. Based on these studies we choose two MAbs for follow-up studies. At this time, we have designed and are in the process of expressing three different recombinant homodimeric constructs with the Fv regions of these three MAbs.

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INTRODUCTION:

The purpose of these studies was to select anti-Her-2 MAbs, which, as homodimers, would most effectively signal apoptosis of Her-2-overexpressing prostate cancer cell lines. This involved generating chemical homodimers of ten monoclonal antibodies (MAbs) and carrying out apoptosis assays using two Her-2-overexpressing prostate cancer cell lines, LNCap. The two homodimers which signaled best were selected for cloning of the Fv regions from the corresponding hybridomas and the generation of recombinant homodimers using three different strategies. Our progress in the generation of these recombinant homodimers is described.

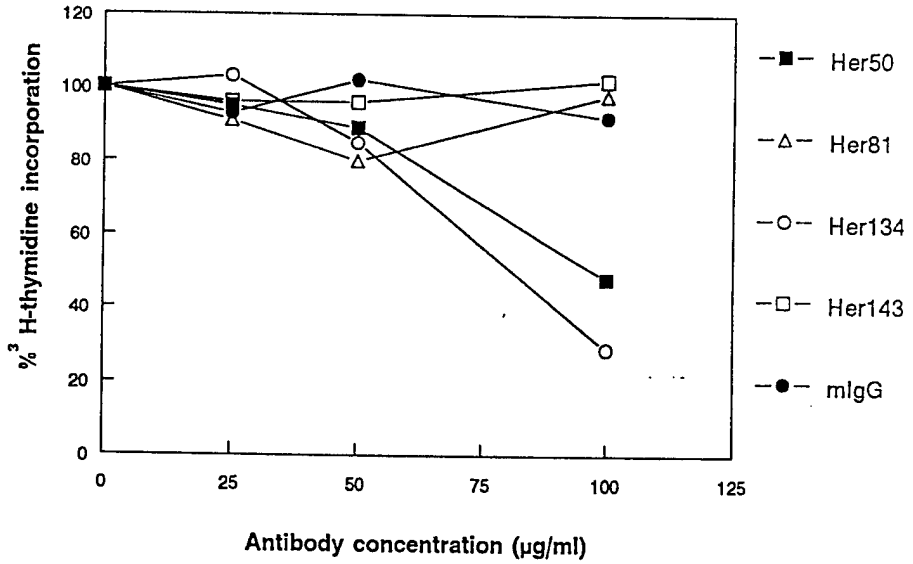
BODY:

Aims 1, 3, 4: Are on hold awaiting completion of Aim 2.

Aim 2: To determine which anti-Her-2 MAb dimers are optimal.

Results:

Her-2-specific dimers inhibit proliferation of LNCapFGC (PSA+) prostate carcinoma cells



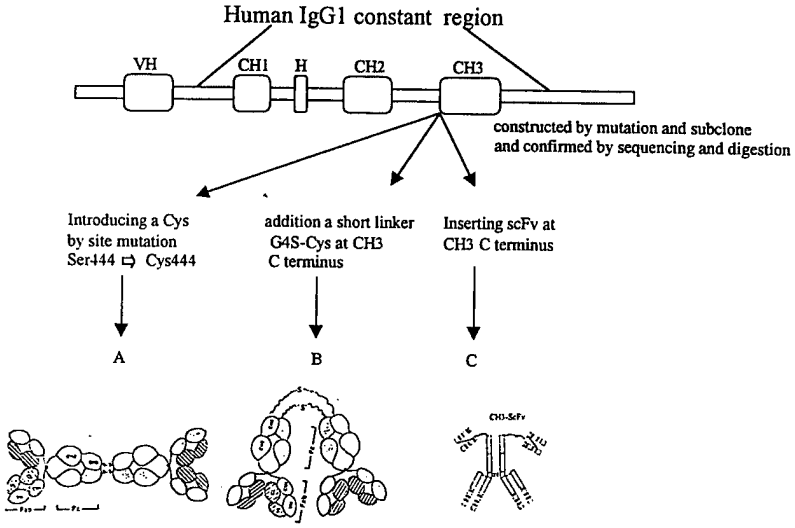
1. As shown in Figure 1, Her50 and 134 most effectively inhibited proliferation of LNCap cells.

Aim 5: To clone and express homodimers from Her50, 66 and 164.

Results:

Mouse/human chimeric antibodies are being generated using the human IgG_1 and kappa constant region domain expression vectors, pAH4604 and pAG4622, kindly supplied by Dr. Sherrie Morrison (1). We are engineering MAb dimers using two strategies which rely on the formation of intermolecular disulfide bonds between engineered cysteine residues (Figure 3).

Figure 2. Homodimeric Constructs



One strategy involves introducing a cysteine residue near the C-terminus of the heavy chain (2,3) (Panel A). While the other adds one or more cysteine residue to the heavy chain using a short flexible linker (Panel B). As an alternate approach, we are making tetravalent MAbs by adding a single chain Fv (scFv) domain to the heavy chain C-terminus using a short linker extension (4,5) (Panel C). We are making site-specific mutations to introduce the cysteine residue, or to create two unique restriction sites for inserting either the cysteine with a linker or the scFv sequence at the C_H3 C-terminus.

Thus far our progress is as follows:

1. Construction A has been finished and cotransfected into drug-marked SP2/0 myeloma cells with a light chain expression vector and helper plasmid pSV2gpt. After selection by histidinol, 42 wells in 288 wells have clones. Among them, six wells were positive for human IgG by ELISA. We are currently subcloning these cells.

1. Construction B is on the last step of building the plasmid.

3. Construction C has been finished and cotransfested into SP2/0 with a light chain expression vector and helper plasmid pSV2gpt. We are currently selecting clones.

The anti-HER-2 mouse heavy chain variable region cDNAs is being cloned from the Her 50 and 134 hybridoma cell lines and inserted into the altered pAH4604s, and the mouse light chain variable domains are similarly being inserted into pAG4622. These will be used to cotransfect

the Ig non-producing myeloma cell line, SP2/0, for simultaneous expression of the two MAb chains and secretion of intact, functional anti-HER-2 chimeric MAb. The yield and monomer/dimer ratio will be determined as well as the stability of the disulfide linked dimers. In addition, the binding avidity and bioactivity of the multivalent MAbs will be compared with IgG monomer and chemically generated dimers.

KEY RESEARCH ACCOMPLISHMENTS:

- The best homodimers have been identified.
- Strategies to clone Fvs from the two selected hybridomas have been developed and experiments are in progress.
- Strategies to prepare homodimers and express them in SP2-0 cells – have been developed and are being evaluated.

REPORTABLE OUTCOMES:

None

CONCLUSIONS:

We have identified two anti-Her-2 homodimers which inhibit proliferation of a Her-2 overexpressing human prostate cancer cell line. Because the generation of chemical homodimers is expensive, gives low yields and several bioproducts we are attempting to generate recombinant homodimers. Three strategies are being attempted. Over the next six months we should know which strategy will work best based on yield, and activity. The most useful construct(s) of the three selected MAbs will then be tested *in vitro* and *in vivo* using a SCID mouse xenograft model which we are beginning to develop.

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APPENDICES:

None