A DIFFERENT METHOD FOR OBTAINING AN ENGINEERED MURINE ANTIBODY, THAT RECOGNIZES EPIDERMAL GROWTH FACTOR RECEPTOR, WITH REDUCED IMMUNOGENICITY

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Introduction

Monoclonal antibody producing hybridomas have been most readily obtained from immunized rodents. In most cases where rodent antibodies have been used for therapy, the recipients have elicited an immune response directed towards the antibody. These reactions have limited the duration and effectiveness of the therapy. The engineered antibodies have been designed to replace as much as possible the xenogeneic sequences with the equivalent human sequences. Chimeric and humanized antibodies are among the genetically-engineered antibodies (1, 2). It is worth noting that even the replacement of the constant regions with human equivalents may not abolish their immunogenicity, still approximately half of the recipients mounts an immune response to the rodent variable regions.

A further procedure for the humanization of an antibody has been suggested by Padlan (3). It is based on the fact that the antigenicity of a protein is dependent on the nature of its surface; therefore, a number of the solvent-accesible residues in the rodent variable region are substituted by residues from a human antibody.

Several groups have developed automated-computarized methods for the identification of sequence features and structural determinants that play a role in the MHC restriction of helper T-cell antigenic peptides (4, 5). Using these algorithms, it has been possible to identify predicted T cell-presented peptides.

Here we describe a new method to reduce immunogenicity through the humanization of the predicted T cell epitopes present in the sequence of the heavy chain variable region of an anti-epidermal growth factor receptor murine monoclonal antibody.

Results and Discussion

The heavy chain variable region sequence of MAb R3 was analyzed for T cell antigenic sequences by using the computer algorithm AMPHI (4), which predicts segments of the sequences of eleven amino acids in length with an amphipatic helix structure, which most likely could bind to MHC II molecules.

Five amphipatic peptides were identified. Two of them involved CDR2 and CDR3 and therefore were not considered for amino acid replacements.

Possible murine T cell epitopes in the framework regions were: H3-H13, H8-H20, H74-H84.

The whole murine heavy chain variable region sequence was compared by homology searching with the human sequences included in the Gene Bank and EMBL databases. A human sequence was found having 75 % homology with the FR regions. Two complementary strategies were followed to reduce immunogenicity by replacing the murine amino acids in some positions, according to the human sequence identified. Two amino acid replacements in FR1: Leu11 by Val and Val12 by Lys, were enough to break the amphipatic helix segments. Four amino acid replacements were required for the complete humanization of the H74-H84 peptide: Ser75 by Thr, Thr76 by Ser, Ala78 by Val, Thr83 by Arg. Computer modeling of the MAb R3 variable region suggested that the proposed amino acid replacements should not have any influence in binding affinity. Positions 11, 12 and 83 were distant from CDRs/FRs interface. Ser75 residue was pointing to the outside; on the other hand, Thr76 was accesible from the top of the molecule, but the substitution by Ser was a conservative change. The replacement of Ala78 by Val should not require steric rearrangements.

We had previously obtained a humanized version of the MAb R3 by CDR-grafting (this volume). The FRs of the human immunoglobulin REI was selected for VK region. NSO myeloma cells were cotransfected with pSV expression vectors containing both the modified quimeric heavy chain and the humanized light chain. The hybrid recombinant antibodies were tested using radio receptor assay for their ability to compete with EGF binding to its receptor, having the same affinity as the original murine antibody (8×10^{-9} M).

Cercopithecus aethiops monkeys (two in each group) were immunized with murine, humanized and mutant hybrid MAb R3. A high IgG response to murine MAb R3 was obtained when this antibody was used as immunogen after two immunizations. A lower but still measurable IgG response (1/10 000 sera dilution) to the murine MAb R3 was obtained when monkeys were immunized four times either with the humanized antibody or with the VH mutant version.

Experiments are in progress with a complete VH and VK mutant version of the quimeric recombinant MAb R3.

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