RESHAPING OF A HUMAN MONOCLONAL ANTIBODY TO EPIDERMAL GROWTH FACTOR RECEPTOR TO RECOVER BINDING AFFINITY

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Introduction

Previous studies of epidermal growth factor receptor (EGF-R) expression in human breast cancer suggest that this receptor is important in the growth regulation of these tumors (1,2). The experimental results have led to the development of the EGF-based blocking immunotherapy concept (3). We have generated a mouse monoclonal antibody (R3) against the EGF-R, which inhibits the EGF binding and the EGF-dependent autophosphorylation of the receptor (4). While this antibody proved to have anti-tumor effects in vivo, as several other anti-EGF-R murine MAbs, results from clinical trials using these antibodies have been disappointing (5, 6).

Humanization by CDR-grafting has been devised in an attempt to reduce the undesired immunogenicity associated with murine MAbs. In this procedure, only the genetic information for the hypervariable complementarity determining regions (CDRs) of the murine antibody is transplanted into human variable region frameworks (7). However, to recover the binding capability of the original antibody, some murine amino acids from the frameworks have to be retained. A comprehensive list of framework residues that may have some influence on the binding site structure has been published by Padlan (8). Nevertheless, it might be necessary to retain other residues not found in this study. In this paper we report the reshaping of a human antibody to have the binding site of the murine MAb R3. A full paper describing all the experimental work will be published elsewhere.

Results and Discussion

Cytoplasmic RNA was extracted from R3 murine hybridoma cells and cDNA was obtained using specific primers for the Ig heavy chain variable region (VH) and kappa light chain variable region (VK). VH and VK were then amplified using the polymerase chain reaction (PCR) and cloned into the M13 vector. Twelve independent clones from two separate cDNA samples were sequenced in both directions. VH and VK were digested and ligated into the pSV-gpt (confering resistance to neomycin) and pSV-hyg (confering resistance to hygromycin) expression vectors (9). These vectors contain human IgG1 and human kappa constant regions, respectively. The chimeric antibody was assayed for its ability to bind to EGF-R using a radio receptor assay (RRA). This antibody inhibited the binding of EGF to its receptor with approximately the same inhibition constant (8 × 10^(-10) M) as the original murine antibody, confirming that the correct mouse variable regions were cloned and that the new antibody isotype did not affect the binding.

To select the human frameworks (FRs) on which to graft the mouse CDRs, the VH and VK regions of MAb R3 were compared with consensus sequences of all subgroups of human VH and VK regions, respectively. The FRs of the human immunoglobulins Eu and RE1 were selected for VH and VK, respectively.

Oligonucleotides were synthesized containing the VH and VK CDRs flanked by short sequences drawn from the selected human frameworks and were subsequently grafted into these FRs by site-directed mutagenesis. Humanized R3 (humR3) VH and VK domains were cotransfected into NOS myeloma cells. Afterwards, mycophenolic acid resistant clones were selected and screened by ELISA for human antibody production. Human IgG1 was detected at levels ranging from 1 to 5 µg/mL. Functional binding of purified humR3 to EGF-R was undetectable. However, when cells were cotransfected with murine R3-VH and humR3-VK, the produced antibody bound to EGF-R, while transfection with the opposite combination did not yield a functional receptor antagonist.

In order to recover the binding capability, several changes were made in the VH framework region of humanized R3 based on previously published work and molecular modeling. Six versions of reshaped R3-VH were constructed, in which different combinations of residues at positions 66, 67, 75, 76 and 93 were mutated back to the original murine amino acids.

A molecular model of the marine R3 variable region was constructed to analyze the possible effects of these mutations.

Experimental data and molecular modeling analysis indicated that the loss of binding for the humanized antibody was due mainly to the substitutions made at positions 76 (Thr→Asn) and 93 (Thr→ Ala) in the original mouse sequence.

The reshaped antibodies, that contain these two residues, showed almost complete recovery of the EGF-binding inhibition properties (K_i ≈ 1.0 × 10^6 M). Residue 76 is located on the top of VH, in a loop that connects two β-sheet strands. This amino acid was not included by Padlan (8) as a critical residue for CDRs conformation.

GANGLIOSIDE VACCINES: ANTI-IDIOPTYPIC MONOCLONAL ANTIBODIES AS ANTIGEN SURROGATES

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Introduction

Gangliosides, which are glycolipids containing sialic acid in their structure, have demonstrated to be important tumor-associated antigens due to their differential expression in tumor tissues in comparison with their normal counterparts (1).

Different immunotherapeutic approaches have been used to obtain an effective immune response against cells expressing defined gangliosides. One of these approaches is the use of anti-idiotypic MAb as antigen surrogates (2, 3).

We have previously described the obtention of an IgM murine MAb, named P3, generated by the immunization of Balb/c mice with purified GM3 (NeuGc) included in liposomes (4). This MAb recognizes the N-glycolyl variants of different gangliosides. Also, P3 MAb reacted with antigens expressed in breast cancer cell lines and malignant human tissues of this origin. In this paper we reported our results in the generation and primary characterization of anti-idiotypic MAb against P3 MAb.

Materials and Methods

Balb/c mice were immunized with two doses (50 μg/dose) of P3 MAb coupled with KLH, in the presence of Freund's adjuvant. Animals with high anti-idiotypic antibody response against P3 MAb (1/50 000) were sacrificed and the spleen cells were fused with the murine myeloma cell line P3X63Ag8.653.

Hybrid culture supernatants were screened by ELISA against different anti-ganglioside IgM MAb. Hybridomas secreting antibodies specific to P3 MAb were selected. Further characterization included the study of their blocking capacity of P3 MAb binding to GM3 (NeuGc) by ELISA, and their ability to generate antibody response against this ganglioside when they were injected couple with KLH and emulsified in Freund's adjuvant in syngeneic and allogeneic mice.

Results and Discussion

Seven IgG1 anti-idiotypic MAb were obtained. All of them reacted strongly with P3 MAb and no reactivity was observed with the other anti-ganglioside IgM MAb tested. The seven anti-idiotypic MAb had the capacity to block P3 MAb binding to GM3 (NeuGc), in a concentration range between 1 to 10 μg/mL.

Five of these anti-idiotypic MAb were capable to elicit a humoral response against GM3(NeuGc) with antibody titers ranged between 1/320-1/1280, in both syngeneic and allogeneic mice, but the specificity of this response differed from P3 MAb recognition due to the reactivity of animal sera not only with N-glycolyl containing gangliosides but also with N-acetyl variants. This anti-ganglioside antibody response generated by the immunization with the anti-idiotypic MAb resembled the humoral responses raised when animals were immunized with GM3(NeuGc) ganglioside included in liposomes.

Ongoing studies are directed to elucidate the capacity of these anti-idiotypic MAb to develop a humoral anti-idiotypic response in xenogeneic models and also, to define their possible anti-tumor effects in vivo.


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