A molecular model of the murine 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 an almost complete recovery of the EGF-binding inhibition properties ($K_i \approx 1.0 \times 10^{-8}$ 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-IDIOTYPIC 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 tumoral 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 MAbs 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 MAbs 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 MAbs. 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 coupled with KLH and emulsified in Freund's adjuvant in syngeneic and allogeneic mice.

Results and Discussion

Seven IgG1 anti-idiotypic MAbs were obtained. All of them reacted strongly with P3 MAb and no reactivity was observed with the other anti-ganglioside IgM MAbs tested. The seven anti-idiotypic MAbs 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 MAbs 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 MAbs 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 MAbs to develop a humoral anti-idiotypic response in xenogeneic models and also, to define their possible anti-tumoral effects *in vivo*.

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