HUMAN ANTI-GANGLIOSIDE MONOCLONAL ANTIBODIES GENERATED BY in vitro IMMUNIZATION

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Introduction

Naturally ocurring antibodies against tumor-associated antigens can be found in humans, mainly in cancer patients (1), but in order to increase the proportion of specific antibody-producing cells, several primary *in vitro* immunization protocols have been succesfully developed.

Immunogenic preparations containing both T- and B-cell epitopes have been used for *in vivo* and *in vitro* immunization systems to stimulate a number of antigen-specific B cells in mice and humans to be further immortalized by fusion or Epstein-Barr virus transformation (2). It has recently been demonstrated that immunization with relevant T helper and B-cell epitopes co-entrapped in an apropriate delivery system such as liposomes is sufficient to overcome the nonresponsiveness to defined B-cell epitopes and even produce an active IgG response (3).

A specific goal addressed by our laboratory has been the production of human monoclonal antibodies (MAbs) to different monosialogangliosides of relevance as therapeutic targets in human tumors such as melanoma, lung and breast carcinomas.

Human MAbs against tumor-associated ganglioside antigens have been obtained by several groups using different approaches, but the generation of human anti-ganglioside MAbs by *in vitro* immunization of human B lymphocytes has not been previously reported.

Material and Methods

Several experiments on *in vitro* immunization of human B lymphocytes from normal donors or melanoma and breast cancer patients were performed using liposomes containing gangliosides as the immunizing antigen, with or without either complete tetanus toxoid or a synthetic T helper epitope derived from tetanus toxin (determinant 830-843). After 6 days in culture, the immunized B cells were immortalized by Epstein-Barr virus infection and the human anti-ganglioside antibody response was evaluated by an indirect ELISA using different mono and disialogangliosides (4). Further characterization of the specificity of the human MAbs was performed by High Performance Thin Layer Chromatography (HPTLC) using a large panel of glicolipids and extracts from different human tumors (5). Immunohistological staining of formalin-fixed paraffin-embedded sections of different malignant melanoma and invasive ductal carcinoma of the breast was performed using the avidin-biotin complex (ABC) technique. The variable region of the heavy and light chain genes were amplified by PCR, cloned into pCRII vector (Invitrogen) and sequenced by the dideoxynucleotide method.

Results and Discussion

Clones producing antigen-specific human antibodies of the IgM isotype against the gangliosides NeuAcGM3, NeuGcGM3 and NeuAcGM2 used as immunogen, were selected from different experiments.Our results indicated that the *in vitro* immunizations were antigen-driven, since the antibody responses specific for the ganglioside used for the immunization, were found. The cultures showing specific antibody production were predominant in the cases where the corresponding ganglioside and the T-helper epitope were incorporated into liposomes and no clones producing anti-ganglioside antibodies were obtained when the antigen was omitted from the cultures.

A number of initially selected clones after few weeks in culture ceased to produce human antibodies. To overcome this widely reported problem concerning the instability of the immunoglobulin production by antibody-secreting cell lines, a method of positive selection using ganglioside-coated magnetic beads (Dynal) has been developed which allowed us to rescue unstable clones.

One of the original clones showing a stable antibody production, identified as IM-11, was further characterized. The binding of the human antibody IM-11 to a large panel of glycosphingolipids sepa-

1. Lloyd KO, Old LJ. Cancer Res 1989; 49:3445-51.
2. Chin LT *et al.* Immunology 1994;81: 428-34.
3. Ifversen P *et al.* Immunology 1995; 84:111-16.
4. Alfonso M *et al.* Hybridoma 1995;14: 209-16.
5. Alfonso M *et al.* Hum Antibod Hybridomas 1995;6:102-12.