Human Immune Response to Anti-Carcinoembryonic Antigen Murine Monoclonal Antibodies

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Abstract

We previously demonstrated that patients with carcinoembryonic antigen (CEA)-producing neoplastic tumors, treated with murine monoclonal antibody to CEA, produced antibodies directed against the constant regions [human anti-mouse antibody (HAMA)] and the idiotypes [anti-Id] of these murine immunoglobulins. In this study, we describe a method for analyzing the presence of such antibodies in the sera of these patients.

The HAMAs were measured by enzyme immunoassay and removed by immunoadsorption on Affi-Gel mouse IgG. The unabsorbed fraction contained the anti-Id antibodies; their presence was demonstrated by binding to the CEA monoclonal antibody (Ab1). The specificity of the binding was assessed by preincubating the sera with Ab1 and measuring the residual nonspecific binding.

When specific binding was detected, the anti-Id antibodies were isolated by adsorption and elution on Affi-Gel Ab1. The anti-Id antibodies were fixed on enzyme immunoassay plates and incubated with a panel of mouse anti-human immunoglobulin to determine their isotypes. In a first series of 24 patients, HAMAs were found in 20 cases and anti-Id antibodies in 19 cases. The isolation of a specific IgG to Ab1 was achieved in 2 cases.

In an ongoing series, the HAMA and anti-Id antibodies were detected in all five patients given injections of another monoclonal antibody to CEA. In two patients an IgG1x anti-Id was isolated from the serum. The potential therapeutic effect of these antibodies is under investigation.

Introduction

MAbs1 to tumor-associated antigens are increasingly used for the diagnosis and the treatment of human carcinomas (1). From a diagnostic point of view, labeled MAbs can allow the localization of tumor tissue. The MAbs have also been used for their cytotoxic activity against the tumor or as carriers of cytotoxic means (chemotherapeutic agents or radionuclides). However, repeated injections of murine immunoglobulins such as MAbs result in the development of HAMA (2-9). The HAMA, by binding to these MAbs, neutralize their active sites and accelerate their removal from the circulation, limiting the effectiveness of the therapy. In addition, these allogeneic MAbs have some degree of toxicity; they might be responsible for allergic reactions (8), serum sickness (7), or renal impairment (10).

We have shown previously (11), as well as others (3-6, 12), that during the course of MAb therapy the host develops antibodies to the variable region of the MAbs, namely anti-Id Abs. These antibodies act in a way similar to the HAMA, preventing the MAb antitumor activity by blocking the binding site of the MAbs to the tumor markers (6-13). Conversely, based on the Jerne’s hypothesis (16), some authors have suggested that anti-Id Abs might exert a beneficial role in antitumor therapy (12, 14, 15).

In this study we used two anti-CEA murine MAbs and we evaluated the patients’ immune response by measuring their production of HAMA and anti-Id Abs. This report will limit itself to the description of the techniques used to identify the antibodies and measure their titers. The effectiveness of the therapy will be reported elsewhere.

Materials and Methods

CEA-MAbs NP-2 and NP-4 are both IgG1, and their preparation and characteristics have been described elsewhere (17, 18). In the first series (Series 1), 24 patients received NP-2; the treatment schedule has been reported previously (11, 19). In a clinical trial currently in progress (Series 2) the patients are treated with 111I-NP-4. The investigation conducted in the first five patients is the subject of this report. Two other CEA-MAbs, NP-1 (control C1) and NP-3 (control C2), recognizing different epitopes on the CEA than NP-2 and NP-4 were used as controls in the assays (17, 18).

The enzyme immunoassay (EIA) for serum HAMA levels measured the antibody response of the patients to the administration of NP-2 or NP-4. Mouse IgG (100 /µg; Pelfrez Biologicals, Rogers, AR) was immobilized on a 96-well microtitration plate (Dynatech Laboratories, Alexandria, VA) at a concentration of 1 µg/well. Another 100 µl of sera diluted in BBS + BSA (BBS containing 1% BSA; Sigma Chemical Co., St. Louis, MO) were added for 1 h at 37°C. The plates were washed and then incubated with 100 µl of peroxidase-conjugated mouse anti-human IgG (Jackson Immunoresearch, Avondale, PA), diluted in BBS + BSA at 37°C. Binding of the peroxidase-conjugated IgG was revealed by the o-phenylenediamine-H2O2 system; the plates were read at 490 mm after addition of 25 µl of 4 N H2SO4. The reciprocal dilution of sera that gave an absorbance reading of 1 in the assay was referred to as the HAMA titer. Anti-Id isolation was conducted at the time of maximum HAMA titers.

The procedure for the isolation of the anti-Id Abs is shown in Fig. 1. Aliquots of sera (0.5 ml) were diluted with an equal volume of BBS and incubated for 2 h at room temperature on a 4-ml column of Affi-Gel 10 (Bio-Rad, Richmond, CA) coupled with 20 mg of mouse IgG. The removal of the antibodies binding to shared determinants among mouse IgG was verified by measuring of the HAMA titer in the unabsorbed fraction (F1). The plasma samples, isolated in F1, were reprocessed a second time when HAMA titer was above 90.

F1 was tested for its capacity to bind in an EIA assay to Ab1 (either NP-2 or NP-4). The wells of EIA plates were coated with the antibodies, 10 µg/ml in PBS, 100 µl/well. The technique was the same as the one used to measure and calculate the HAMA titers (Fig. 2).

In order to define the specificity of the binding of F1 on Ab1, F1 was incubated with Ab1, C1, and C2. Then, the residual binding capacity was measured. We selected a dilution of F1 at which the effects of the inhibitors will be detected even in trace amounts (dilutions giving an absorbance reading of 1.5 to 1.8 in the anti-Id assay). Aliquots of diluted F1 (120 /µl) were incubated with Ab1 and control antibodies, 6, 4.5, 3, 1.5, 0.75, 0.25, and 0 µg in 30 µl of PBS, for 90 min at 37°C. An aliquot of the incubated solution (100 /µl) was then tested in the anti-Id EIA assay on plates precoated with Ab1. The inhibition was calculated for each antibody concentration (Fig. 3).

When an inhibition for Ab1 of 85% or more was achieved, without any inhibition for the controls, anti-Id Ab (Ab2) isolation proceeded. F1 was passed at a flux of 5 ml/h through a 4-ml column of Affi-Gel where Ab1 had been coupled to the matrix (5 mg/ml). The bound fraction consisted of blood circulating CEA and Ab2 and was eluted by...
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**Measurement of HAMA Response.** In the 29 patients studied, 25 developed HAMA titers ranging from 180 to $4 \times 10^5$ (Table 1).

**Measurement of Anti-Id Response.** Before measuring the anti-Id Abs, the HAMA were removed. In most cases one adsorption on Affi-Gel with mouse IgG was sufficient to lower their titer to negligible levels (50 or lower). In 11 patients, the HAMA titers remained elevated. Two successive adsorptions were performed, but if the titers dropped somewhat, in 8 cases binding to mouse IgG could not be completely eliminated (Table 1). Once the HAMA were removed, anti-Id Abs were detected by the EIA in 26 of the 29 patients investigated. The titers ranged from 120 to $1 \times 10^5$ (Table 2). The observed binding was not due to CEA since in this assay the peroxidase-conjugated mouse anti-human immunoglobulin will not bind to CEA but only to a human immunoglobulin. In the first series (Series 1), 21 of 24 sera demonstrated a strong binding to Ab1. In the second series (Series 2) binding to Ab1 was measured in the 5 sera investigated.

The specificity of the anti-Id Abs binding to Ab1 was evaluated using an inhibition assay where F1 was preincubated with Ab1, C1, and C2. The dilution of F1 was chosen accordingly with the results of the direct binding assay (Fig. 2) to ascertain

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**Table 1** HAMA titers before and after adsorption on mouse IgG

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**Table 2** Mouse anti-human (IgM, IgD, IgG, IgG1, IgG2, IgG3, IgG4, IgA, IgA1, IgE, light chain κ and λ), in BBS + BSA, were added and incubated for 20 h at 4°C. (Mouse anti-human globulins were obtained from Southern Biotechnology, Birmingham, AL.)

After extensive washings, peroxidase-conjugated goat anti-mouse immunoglobulin, diluted in BBS + BSA, was added onto the plates for 24 h at 4°C. Binding was then revealed by the o-phenylenediamine-H$_2$O$_2$ system.
that any minimal decrease in the amount of anti-Id Ab which bind to Ab1 would be detected.

The binding to Ab1 was found to be variably inhibited by the preincubation with Ab1 (Tables 2 and 3). In 25 sera the inhibition of the binding was at least 30%. In no circumstance could the binding to Ab1 be inhibited by preincubation with the controls C1 and C2 (Fig. 3). Thus, this demonstrated the specificity of the anti-Id Abs.

When the inhibition of the binding to Ab1 by preincubation with Ab1 was at least 85%, anti-Id Abs were isolated by immunoadsorption. This isolation was done using an Affi-Gel-Ab1 column; this column is able to bind 2 different components, the anti-Id Abs and the human circulating CEA. The isolated anti-Id Abs were isotyped by an EIA.

In the first series of 24 patients, 6 sera showed an inhibition of at least 85%, and who were treated with CEA-MAb NP-2 and NP-4 developed a strong immune response to murine immunoglobulins. The production of HAMA reflects the response to the constant region of the murine IgG and was detected in 25 of the 29 patients studied; its titers ranged from 180 to 4 x 10^5 units. The antiidiotype antibodies (anti-Id) reflect the response to the variable region of the murine immunoglobulin and were measured in 27 of the 29 patients; their titers ranged from 100 to 1 x 10^5 units.

Methods to identify anti-Id antibodies in patients treated with murine MABs have been published previously. In an earlier report of this laboratory (11), we described a blocking assay that measures the ability of the patients' sera to interfere with the binding of radioiodinated F(ab')2 antibody to CEA. In this study, anti-Id antibodies were detected in 5 out of 10 patients. Herlyn and associates developed an assay that evaluates the inhibition of the binding of ^125I-labeled goat or rabbit anti-Id antibodies to their CO17-1A MAB by the anti-Id antibodies present in the patients’ sera (20). They detected a positive response in 35 of 41 patients tested. More recently, Tsujisaki (21) proposed a sandwich assay in which the anti-Id antibodies bound to the MAb are revealed by incubation with ^125I-labeled MAb.

The major advantage of our technique is its simplicity. All the reagents used are commercially available. It is simpler than our previously described method since it does not require the preparation of F(ab')2; fragments of monoclonal antibodies. Furthermore, our technique can be applied to the detection of antiidiotype antibody to murine immunoglobulins other than IgG1 from which the preparation of F(ab')2; fragments is difficult (22). In contrast to the competitive assay it does not involve the preparation of rabbit or goat anti-Id antibodies.

Once the HAMA has been removed by adsorption of the sera on mouse IgG, the specificity of the assay can be assessed by measuring the inhibition of the binding on Ab1 by preincubation with Ab1. In all cases except two, the binding was inhibited by at least 30%. The binding is specific for the anti-Id and the assay if not affected by the level of CEA in the sera.

In conclusion, anti-Id antibodies were detected in most of the patients treated by CEA-MABS NP-2 and NP-4. We isolated and characterized anti-Id antibodies successfully in 5 patients; in the first series, an IgG was identified in 3 cases, while we found an IgG1k in 2 patients of the second series. The significance of these observations requires further investigation.

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References


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