

## Antitumor Immune Response and Interleukin 2 Production Induced in Colorectal Cancer Patients by Immunization with Human Monoclonal Anti-Idiotypic Antibody<sup>1</sup>

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### Abstract

The immunogenicity of human anti-idiotypic antibody has been investigated using a human monoclonal anti-idiotypic antibody (105AD7) which interacts with the binding site of 791T/36, a mouse monoclonal antibody against gp72 antigen. This antigen is frequently expressed in gastrointestinal cancer; therefore, six patients with advanced colorectal cancer have been immunized with 105AD7 as an aluminum hydroxide gel precipitate in a phase I clinical study. Cryopreserved blood mononuclear cells were tested for *in vitro* proliferative responses by [<sup>3</sup>H]thymidine incorporation; plasma samples were tested by enzyme-linked immunosorbent assay for anti-anti-idiotypic and antitumor antibodies, and for interleukin 2. Proliferative responses to gp72 positive tumor cells were seen in four of five patients tested; parallel *in vitro* responses to 105AD7 anti-idiotypic antibody were seen in most of these patients. Interleukin 2 was detected in the plasma of four of six patients after 105AD7 immunization, with peak levels up to 7 units/ml. No toxicity related to anti-idiotypic immunization and no antitumor or anti-anti-idiotypic antibodies were seen. This study shows that human monoclonal anti-idiotypic 105AD7 is immunogenic in cancer patients, inducing cellular antitumor responses and interleukin 2 production. This suggests that human monoclonal anti-idiotypic antibodies may have considerable potential for immunotherapy of human cancer.

### Introduction

An anti-idiotypic antibody which interacts specifically with the binding site of an antibody to a tumor associated antigen may have functional similarity to that antigen, and immunization with an anti-idiotypic antibody could therefore be used to induce antitumor responses. This approach is quite distinct from the use of the variable region of immunoglobulin as a tumor cell surface target for passive immunotherapy in B-cell malignancy. Immunization with anti-idiotypic may induce immune responses not seen after immunization with conventional antigens and can break tolerance to poorly immunogenic antigens (1, 2); anti-idiotypes may also act as substitutes for antigens which are difficult to identify, isolate, or synthesize, and for all these reasons, antiidiotypic immunotherapy is an attractive approach in the tumor context. Until now, anti-idiotypic antibodies raised in goats and mice have been used for this purpose. Although species differences could act as carrier determinants, making the anti-idiotypic more immunogenic, antispecies immunoglobulin reactivity may dominate the response. During the immune responses to antibodies used for tumor localization or targeting of toxin moieties, the patient

may produce antibodies to constant determinants and the variable region (idiotypic) of the antitumor antibody (3, 4), and human monoclonal antibodies with idiotype and nonidiotype binding characteristics can be developed from these patients' lymphocytes (5, 6). We wished to investigate whether human anti-idiotypic antibodies would be immunogenic in cancer patients and developed a human monoclonal anti-idiotypic antibody (6) which is able to prime cellular antitumor responses in rats and mice (7). We have begun a phase I clinical study to investigate the immunogenicity of this human monoclonal anti-idiotypic antibody in patients with advanced colorectal cancer, as well as the safety and toxicity of this approach.

### Patients and Methods

#### Patients

Six patients have been recruited into the study. All have recurrent colorectal carcinoma which is not amenable to treatment; all have multiple hepatic metastases. The patients' ages range from 43 to 75 years (median age, 70 years), and there are two women and four men. Total survival time and recurrence free interval are shown in Table 1; of the six patients, two are still alive (patients 1 and 6). The site of primary tumor, tumor stage (Dukes' classification), and tumor histological grade are also shown in Table 1.

#### Materials

**Monoclonal Anti-Idiotypic Antibody.** Human monoclonal antibody 105AD7 is an IgG1 that interacts specifically with the binding site of 791T/36 mouse monoclonal antibody (6). 791T/36 binds to gp72 antigen expressed on 791T cultured tumor cells (8) and primary colon carcinoma cells (9). 105AD7 was obtained by fusion of lymphocytes from a patient given 791T/36 for immunoscintigraphy (6), and was produced *in vitro* from defined seed lots according to CRC/NIBRC guidelines (10). 105AD7 antibody was purified by affinity chromatography on immobilized 791T/36, sterilized by filtration, heat treated at 60°C for 30 min, and formulated as skin test doses of 10 µg antibody in 0.1 ml, or immunization doses of 100 µg antibody on aluminum hydroxide gel (Alhydrogel 85, provided by SuperPhos Biosector a/s, Vedbæk Denmark). Samples passed tests for pyrogenicity, sterility, *Mycoplasma* contamination, and toxicity (Safepharm Laboratories Ltd., Derby, United Kingdom).

#### Methods

**Clinical Protocol.** This study was approved by the local Ethical Committee, and appropriate patient consents were given. Patients received 10 µg antibody as a skin test to monitor for preexisting hypersensitivity and 24 h later were given injections of 100 µg antibody i.m. as an aluminum hydroxide gel precipitate. A second skin test dose of 10 µg antibody was given 2 weeks after immunization. Venous blood

Received 7/17/91; accepted 8/5/91.

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<sup>1</sup> Supported by grants from the Cancer Research Campaign, United Kingdom.

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Table 1 Patients' clinical status

Patient	Survival (mos)	Recurrence free interval (mos)	Primary site	Stage <sup>a</sup>	Grade <sup>b</sup>
1	>37	7	Cecum	C	Moderate
2	18	5	Cecum	C	Moderate
3	12	0	Sigmoid	C	Poor
4	4	0	Rectum	C	Well
5	9	0	Rectum	B	Well
6	>41	33	Rectum	C	Moderate

<sup>a</sup> Dukes' classification.<sup>b</sup> Histological grade determined by standard criteria.

samples were taken into preservative-free heparin 1 week before and immediately before first administration of anti-idiotypic antibody and then at weekly intervals for 6 weeks after immunization. Blood samples were separated on Lymphoprep (Flow Laboratories, Irvine, Scotland) and mononuclear cells were frozen in liquid nitrogen using dimethyl sulfoxide as a cryopreservative. Plasma samples were stored frozen at  $-70^{\circ}\text{C}$  in small aliquots.

Toxicity studies were carried out daily on an inpatient basis for the first week following immunization and weekly on an outpatient basis for the succeeding 5 weeks. Vital signs and physical condition were monitored. Electrocardiograms and radiography were performed prior to immunization, after the first week, and at the conclusion of the 6 weeks. Blood count and differential, serum electrolytes, and urine blood and protein content were analyzed daily for the first week and weekly thereafter. Liver enzymes, serum proteins, complement components, and circulating immune complexes (anti-complementary activity) were analyzed weekly.

**Detection of Antibodies in Plasma.** Tests for antibodies binding to 105AD7 and to 791T tumor cells were carried out using ELISAs<sup>3</sup> by previously described techniques (4).

**Interleukin 2 (IL-2) in Plasma.** Plasma samples were tested for IL-2 using a sandwich ELISA with highly purified rabbit anti-IL-2 antibodies as the capture and detecting antibodies (Dupont, Ltd.). This assay has been shown not to cross-react with interleukin  $1\alpha$ , interleukin  $1\beta$ , interleukin 3, interleukin 4, colony-stimulating factor 1, tumor necrosis factor  $\alpha$ , and  $\gamma$  interferon or rheumatoid factor.

**Lymphocyte Proliferation Assays.** Blastogenesis experiments were carried out on a time course set of cryopreserved blood samples tested in parallel. Mononuclear cells ( $5 \times 10^4$ ) were cultured with  $3 \times 10^3$  irradiated (5000 R) 791T tumor cells or  $10 \mu\text{g}$  105AD7 antibody; controls included irradiated Colo205 tumor cells (gp72 negative), normal human IgG, and medium alone. Tritiated thymidine was incorporated over the last 24 h of a 5-day culture period. The proliferation index was calculated as a multiple of the incorporation of tritiated thymidine after stimulation relative to the respective medium controls. The statistical significance of the stimulation was estimated using analysis of variance and the modified least significant difference method to allow for multiple comparisons (SPSS/PC; SPSS Inc., Chicago, IL). All the stored mononuclear cell preparations used in this study proliferated strongly in response to phytohemagglutinin, and mononuclear cells from a normal individual tested in parallel with the patients' mononuclear cells did not respond to any of the stimulator cells or antibodies used (data not shown).

## Results and Discussion

Six patients have been immunized with human anti-idiotype antibody 105AD7, with a total of 15 immunization cycles, each consisting of skin test with  $10 \mu\text{g}$  105AD7, immunization with  $100 \mu\text{g}$  105AD7 or aluminum hydroxide gel, and a second skin test of  $10 \mu\text{g}$  105AD7. Individual patients have received up to four immunization cycles. No evidence of toxicity induced by human monoclonal antibody immunization was seen. Analysis

<sup>3</sup> The abbreviations used are: ELISA, enzyme-linked immunosorbent assay; IL-2, interleukin 2; i.d., intradermal.

of plasma samples for antibodies binding to 105AD7 (by their ability to block the binding of 791T/36 to 105AD7) and to 791T tumor cells which express high levels of gp72 antigen, revealed no anti-anti-idiotype or antitumor antibody responses to immunization with 105AD7.

Proliferative responses to irradiated tumor cells of blood mononuclear cells from patients immunized with the human monoclonal anti-idiotype antibody 105AD7 were seen in 4 of 5 patients for whom lymphocytes were available; the time course of these responses is illustrated by patients 1, 3, and 5 (Fig. 1). Lymphocytes from patient 1 showed a significant

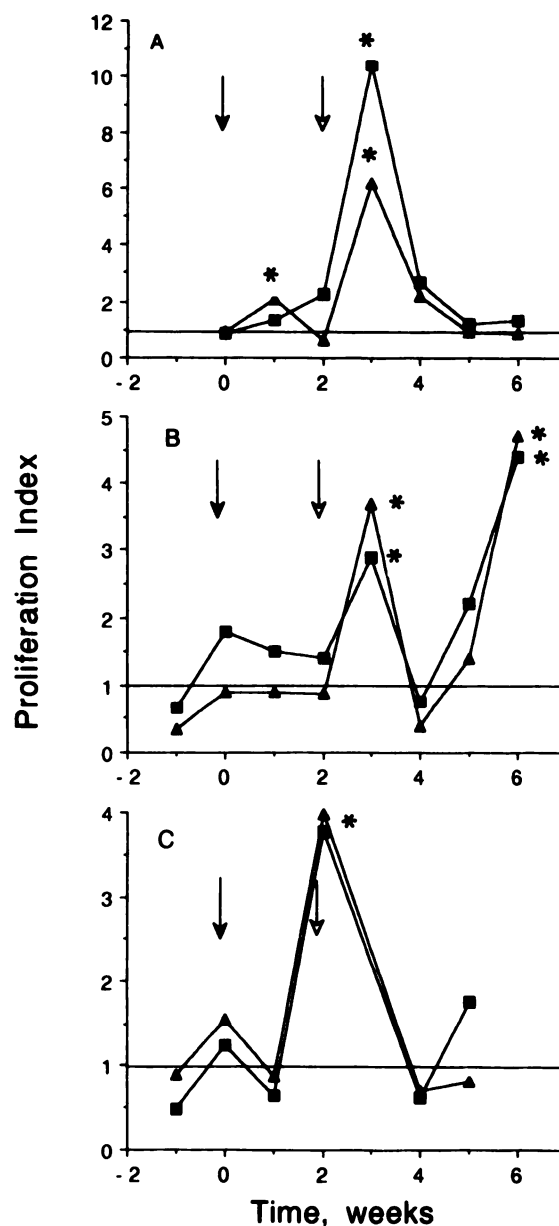


Fig. 1. Time course of blastogenic response to irradiated tumor cells or anti-idiotype in patients 1 (A), 3 (B), and 5 (C). Cryopreserved blood mononuclear cells taken before (-1, 0) and after (1 to 6) immunization with 105AD7 anti-idiotype stimulated with gp72 positive tumor cells (791T; ■) or 105AD7 antibody (▲). Closed arrow, skin test with  $10 \mu\text{g}$  105AD7 antibody followed by immunization with  $100 \mu\text{g}$  aluminum hydroxide gel precipitated 105AD7 antibody; open arrow, skin test with  $10 \mu\text{g}$  105AD7 antibody alone. Proliferation determined by [ $^3\text{H}$ ]thymidine incorporation for the last 24 h of a 5-day culture. Proliferation index, dpm with stimulator/dpm in medium controls. \*,  $P < 0.05$  using the modified least significant difference method.

Table 2 Summary of patient responses to immunization with 105AD7 human monoclonal anti-idiotypic antibody

Patient	Proliferation after stimulation with <sup>a</sup>				Plasma IL-2 <sup>b</sup>
	791T	Colo205	105AD7	HulgG <sup>c</sup>	
1	10.4 <sup>d</sup>	NA	6.2 <sup>d</sup>	NA	7
2	1.3	1.8	1.1	1.4	ND
3	7.3 <sup>d</sup>	1.8	5.8 <sup>d</sup>	2.1	4
4	3.1 <sup>d</sup>	0.8	1.2	1.5	7
5	3.8 <sup>d</sup>	1.2	4.0	1.8	ND
6	NA	NA	NA	NA	6

<sup>a</sup> Maximum proliferation index, calculated as the multiple of [<sup>3</sup>H]thymidine incorporation with respect to corresponding medium alone control.

<sup>b</sup> Maximum plasma level of IL-2, units/ml, by ELISA assay.

<sup>c</sup> HulgG, normal human IgG; NA, not available; ND, not detectable.

<sup>d</sup>  $P < 0.05$  by modified least significant difference method.

proliferative response to 791T cells 2 and 3 weeks after immunization, which then declined; preimmunization and 1 week postimmunization samples showed no response. The strongest response to 105AD7 was also seen 3 weeks after immunization, but in this case, a significant response was seen at the 1-week time point. Patient 3 responded to 791T tumor and to 105AD7 at the 3-week time point; interestingly, this patient also responded at 6 weeks after alum immunization (Fig. 1B). Again, the response to anti-idiotype paralleled his response to gp72 positive tumor, with significant responses 3 and 6 weeks after immunization. Similarly, lymphocytes from patient 5 responded to 791T 2 weeks after immunization (Fig. 1C).

The maximum blastogenic response of all the patients is summarized in Table 2. No responses to Colo205 cells, which do not express the gp72 antigen, or to normal human IgG were seen throughout the monitoring period after this first immunization (maximum proliferation index, 1.8 and 2.1, respectively). Patient 2 showed no significant blastogenic responses to 791T tumor cells or 105AD7 anti-idiotype antibody throughout the monitoring period. Patient 4 responded to 791T tumor cells and not to Colo205 cells, but in this case, there was no response *in vitro* to 105AD7 antibody. Unfortunately mononuclear cells were not available from patient 6 because of a freezer failure.

Thus, treatment of colorectal cancer patients by injection with monoclonal human anti-idiotype in the form of an aluminum hydroxide precipitate produced a time dependent specific response to cultured tumor cells in a blastogenesis assay. A proliferative response was not observed with patients' lymphocyte samples taken before immunization, showing that the allogeneic tumor cells used were not nonspecifically mitogenic and were not inducing a primary response *in vitro* during the culture period. The selective response to gp72 positive tumor cells in patients 3, 4, and 5 also indicates that a specific recognition event has been stimulated by immunization with anti-idiotype antibody. The nature of this recognition is not clear and is the subject of further study. Processing of tumor cell derived products by major histocompatibility complex class II antigen expressing antigen presenting cells followed by triggering of CD4 positive T-cells is a possible mechanism. Similar arguments apply to the *in vitro* responses observed to 105AD7 antibody, which largely paralleled the responses to gp72 positive tumor cells. The relationship between the response to anti-idiotype and tumor cells is an interesting and important one. We plan to investigate this relationship at the responder cell level by developing T-cell lines and clones from anti-idiotype immunized patients.

IL-2 levels in plasma of anti-idiotype immunized patients were tested by ELISA as an index of *in vivo* T-cell activation. IL-2 was detected in the postimmunization plasma of 4 of 6

patients; the highest plasma levels achieved in each patient are summarized together with respective blastogenesis responses in Table 2. Differing patterns of IL-2 production after repeated anti-idiotype immunization are illustrated in Fig. 2. Patient 1 (Fig. 2A) produced a short-lived response promptly after each immunization, whereas patient 3 (Fig. 2B) exhibited a much more prolonged response to second and third cycles of immunization. For each of these cycles, IL-2 was detected in five consecutive weekly plasma samples, with peak levels of 4 units/ml. The kinetics of IL-2 production has a biphasic appearance during these immunization cycles, with a secondary peak apparently in response to the skin test dose of 105AD7 administered 2 weeks after injection of aluminum hydroxide gel precipitated 105AD7. A similar phenomenon was seen with patient 6 (Fig. 2C), where more IL-2 was present following the skin test than following the aluminum hydroxide gel precipitated antibody. IL-2 was not detectable at other time points before or up to 6 weeks after immunization.

The production of sufficient IL-2 to allow its ready detection in plasma following immunization with human anti-idiotype antibody is a significant finding, suggesting that a brisk T-cell

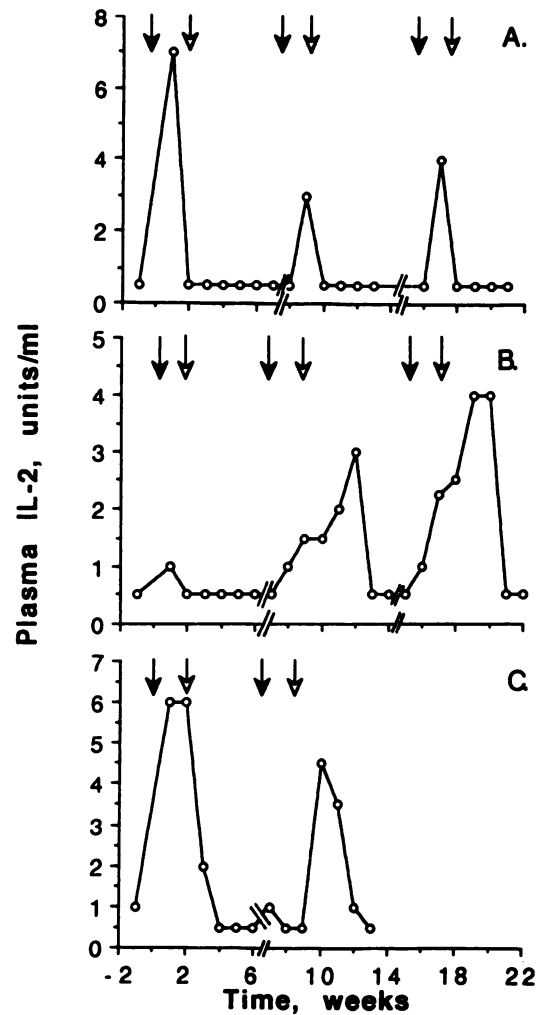


Fig. 2. Plasma IL-2 levels after repeated immunization with 105AD7 human anti-idiotype antibody. Plasma IL-2 determined by ELISA, in units/ml for patient 1 (A), patient 3 (B), and patient 6 (C). Closed arrow, skin test with 10  $\mu$ g 105AD7 antibody followed by immunization with 100  $\mu$ g aluminum hydroxide gel precipitated 105AD7 antibody; open arrow, skin test with 10  $\mu$ g 105AD7 antibody alone.

response is induced. Pharmacokinetic studies of IL-2 suggest that natural and recombinant lymphokines are cleared from the circulation quite rapidly (11). Patients with advanced cancer have previously been shown to have low resting levels of IL-2 (12), and it has been shown that continuous infusion of 3000 units/kg/h recombinant IL-2 results in serum levels of 5 to 10 units/ml (13). In this context, the levels of IL-2 detected and the sustained kinetics observed in some patients indicates that a significant immune response is being induced by immunization with anti-idiotype antibody.

The kinetics of the plasma IL-2 response in patients 3 and 6 suggests that the antibody injected alone as a skin test challenge could be contributing as an immunogen. The biphasic response in the blastogenesis assays with patient 3 (Fig. 1B) could also be ascribed to responses to the initial immunization and then the skin test challenge. However, the response is variable between patients, with patient 1, for example, seeming to show only a brief elevation of plasma IL-2 following administration of aluminum hydroxide gel precipitated antibody. More detailed studies of dosing schedules are therefore being planned, to investigate both higher and lower immunization doses than those used thus far.

Although evidence of cellular responses to immunization was obtained in blastogenesis assays and plasma IL-2 analyses, it is noteworthy that skin test responses were not observed to i.d. challenge with 105AD7 antibody. This may be because anti-idiotype antibody is not retained and processed appropriately at this site; the time of challenge may also not have been appropriate. A further consideration is that T-cells may have been recruited to other sites and therefore are not available to extravasate and initiate a response at the site of challenge.

Polyclonal and monoclonal anti-idiotype antibodies raised in other species have thus far been investigated for immunotherapy of human solid tumors (14, 15). Goat anti-idiotypic antibodies to mouse monoclonal antibody 17-1A have been used to immunize patients with colorectal cancer (14), with evidence of cellular infiltration of metastatic lesions (16). As indicated above, species incompatible antibody constant regions may act as carrier determinants and increase the immunogenicity of the idiotypic, but anti-species immunoglobulin responses may dominate the response, especially after repeated immunization. Human anti-idiotype should not induce such anti-species responses, but it is arguable that human antibody may not be sufficiently immunogenic to induce an antitumor response. This view is not consistent with the suggestion that antitumor immune responses could be generated spontaneously in patients making anti-idiotype responses to injected antibodies, which may account for the delayed antitumor effects sometimes observed after administration of antibody containing immunotoxins (17, 18). There is considerable evidence that cellular rather than antibody responses are more effective for tumor rejection (19), and the induction of cellular responses to anti-idiotype may be favored by a less immunogenic presentation.

We show here the development of a blastogenic response to tumor and the appearance of IL-2 in the plasma after immunization with a human monoclonal anti-idiotype antibody administered without conjugation to a carrier determinant and using aluminum hydroxide gel as a mild adjuvant. These findings suggest that human monoclonal anti-idiotype antibodies are sufficiently immunogenic to induce T-cell responses in cancer patients, without the disadvantages of antispecies immunoglobulin responses involved in the use of nonhuman anti-idiotype. Human anti-idiotype antibodies can be derived by

hybridoma techniques as in the present study, or perhaps more conveniently by repertoire cloning or humanization of xenogeneic anti-idiotype antibodies (20). Immunization with these human or humanized anti-idiotype antibodies could be an effective and importantly a nontoxic method of inducing immune responses to tumor cells.

#### Acknowledgments

We are most grateful to Superphos Biosector a/s for Alhydrogel 85 aluminum hydroxide gel used to formulate immunization doses of 105AD7 antibody; to Dr. M. R. Price for help with antibody purification; and for the skilled technical assistance of D. S. Betts, M. Mee, R. Moss, and M. Sekowski. We are also grateful to Professor H. Sewell, Department of Immunology, Nottingham University, for helpful discussions.

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