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Advances in Brief

Enhanced Cell-mediated Tumor Killing in Patients Immunized with Human Monoclonal Antiidiotypic Antibody 105AD7[1]

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Abstract

A human antiidiotypic monoclonal antibody (105AD7) has been shown to induce antitumor cellular responses in animals and appears to prolong survival in patients with metastatic colorectal cancer without associated toxicity. Proliferative leukocyte responses to the targeted tumor antigen gp72 were observed in these patients and plasma interleukin 2 levels were increased following immunization. Autologous tumor tissue was not available in these patients, so antitumor cytotoxicity could not be measured. This issue has now been addressed in an adjuvant clinical study in primary rectal cancer patients.

Six patients with rectal cancer were immunized preoperatively with 105AD7. Peripheral blood lymphocytes taken prior to immunization were tested against tumor cells extracted from biopsies also obtained prior to immunization or from natural killer (NK)-sensitive target cells. Cryopreserved lymphocytes taken before and after tumor immunization, fresh peripheral blood lymphocytes taken immediately prior to surgery, and lymphocytes from tumor-draining lymph nodes were tested against autologous cells from the resected specimen or NK-sensitive target cells. Significant killing of autologous tumor cells, which was not due to NK activity, was seen with cryopreserved lymphocytes or lymph node cells of three patients at 1–2 weeks postimmunization with 105AD7 but not on pretreatment biopsies. Enhanced NK activity was seen 2–3 weeks postimmunization in 3 of 6 patients. These results indicate that 105AD7 human monoclonal antibody immunization enhances cytotoxicity in rectal cancer patients by specific and nonspecific effector mechanisms.

Introduction

Immunization with antiidiotypic monoclonal antibodies may offer an alternative immunological approach to tumor therapy. A human monoclonal antiidiotypic antibody, 105AD7, which mimics a colorectal tumor associated antigen, gp72, has been developed (1). 105AD7 induced delayed hypersensitivity responses to gp72-positive human tumor cells in mice and rats (2) and was therefore a candidate immunogen for idiotypic immunotherapy of colorectal cancer patients. Phase I clinical studies in advanced colorectal cancer patients showed that immunization with 105AD7 is nontoxic. Immunized patients showed evidence of T-cell blastogenesis responses to gp72 expressing cells and enhanced IL-2[3] production (3) but without antibody induction. Moreover, patients in the 105AD7 study had brisk and significant lymphocytosis and an increase in survival when compared with a contemporary group of patients treated in the same center (4). These encouraging results are currently being confirmed in a double-blind randomized study in a similar cohort of patients. Several T-cell mechanisms could account for the apparent delayed progression of tumor growth after 105AD7 immunization. These included specific and nonspecific activation of MHC restricted regulatory and effector T-cells and activation of other nonspecific effector mechanisms. These issues have been addressed in an adjuvant clinical study in primary rectal cancer patients.

Materials and Methods

Patients. Six patients have been recruited into the study. All have primary rectal cancer. The ages of patients ranged from 60 to 79 years and there are 4 men and 2 women. The tumor stage (Duke's classification) and tumor histological grade are shown in Table 1.

Monoclonal Antiidiotypic Antibody. Clinical grade monoclonal antibody 105AD7 was produced as described previously (3) using the guidelines of the Cancer Research Campaign (5). Samples of the seed lots passed testing for sterility and viral contamination. Antibody for clinical use was prepared as either 10 μg of antibody in sterile saline for intradermal skin test doses or as alum-precipitated i.m. doses of 100 μg of antibody/ml.

Clinical Protocol. This study was performed under the auspices of the Cancer Research Campaign, United Kingdom Phase I/II clinical trials committee which was approved by the local Ethical Committee, and appropriate patient consents were given. Patients received 10 μg of 105AD7 antibody as a skin test to monitor for preexisting hypersensitivity; 48 h later, patients with a negative skin test were given i.m. injections of 100 μg of antibody as an aluminum hydroxide gel precipitate. Tumor biopsies were taken at endoscopy prior to immunization and tumor was obtained immediately postresection to provide a source of extractable target cells (6). Venous blood samples were taken into preservative-free heparin 1 week before first administration of antiidiotypic antibody and then at weekly intervals until tumor resection. Blood samples were separated on Lymphoprep (Flow Laboratories, Irvine, Scotland) and mononuclear cells were frozen in liquid nitrogen using dimethyl sulfoxide as a cryopreservative.

Cytotoxicity Assays. Cytotoxicity experiments were carried out on either fresh PBMC against tumor cells obtained from the preimmunization biopsy and then against tumor cells obtained from the resected tumor or on a time course set of cryopreserved blood samples. Cryopreserved lymphocytes were defrosted and grown in the presence of both defrosted and irradiated autologous biopsy extracted tumor cells and IL-2 for 3 days and then tested for cytotoxicity against freshly disaggregated tumor cells from the resected specimen. NK activity was also tested in parallel on all mononuclear cell samples using K562 cells as targets. This assay was also validated using fresh mononuclear cells obtained from a normal donor. Varying numbers of effector cells were added to 10^4 chromium-labeled (10^6 cells labeled with 100 μCi of [^{51}Cr]-chromium for 45 min at 37°C) target cells (freshly disaggregated tumor cells or K562 cells to produce ratios ranging from 3:1 to 50:1). There were considerable constraints on the ratio of effector to target cell assays which could be performed due to the amount of blood that could be taken ethically on a weekly basis prior to surgery and also to the yield of viable cancer cells obtained from the biopsy or resected specimen. This was a particular problem with the resected specimen because the kinetic studies were all tested against this sample and there were up to 8 effector cell populations in a single assay. It proved impossible to obtain sufficient viable tumor cells from the tumors of patients PT08 and PT10 to perform the autologous tumor assays. Chromium release was measured in 100 μl of supernatant at 4 and 18 h. The percentages of ^{51}Cr release and cytotoxicity were calculated, and the relationship between the percentage of cytotoxicity and effector cell numbers was fitted using an exponential equation. Lytic units per 10^6 effector cells were calculated, defin-

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[3] The abbreviations used are: IL-2, interleukin 2; NK, natural killer; CTL, cytotoxic T-lymphocyte; PBMC, peripheral blood mononuclear cells; MHC, major histocampatibility complex; HLA, histocompatibility at locus A.

Table 1 *Clinical status of patients*

	Clinicopathology			HLA type					Treatment		Immune response	
Patient	Site	Stage[a]	Grade	MHC class I			MHC class II		Immunization[b] (weeks)	Operation[c]	Autologous cytotoxicity[d]	NK[e]
PT03	Rectum	B	Mod[f]	A1, 9^{24}	B8, w41	Cw7	Dr3, w6, w10	DQw1, w2	0, 16, 40	3	+	+
PT04	Rectum	A	Mod	A1, 2	B8, 12^{44}	ND	Dr3, 4	DQw2, w3	0, 14, 39	6	+	−
PT06	Rectum	C	Mod	A1, 29	$B12^{44}$, 15^{62}	ND	Drw6, 5^{12}	DQw1, w3	0, 14, 38	3	−	−
PT08	Rectum	A	Mod	$A9^{24}$, 11	$B15^{62}$	Cw3	Dr4, 5^{11}	DQw3	0, 13, 37	2	ND	−
PT09	Rectum	B	Mod	$A9^{24}$, 11	B27, 37	ND	Dr1, 2	DQw1	0	2	+	+
PT10	Rectum	B	Mod	ND	ND	ND	ND	ND	0	3	ND	+

[a] Modified Duke's classification.
[b] Patients are immunized with 105AD7 monoclonal antibody at 10 μg intradermal and 48 h later 100 μg i.m.
[c] Weeks after initial 105AD7 immunization that rectal tumor was resected.
[d] Autologous cytotoxicity was measured by chromium release from freshly disaggregated autologous tumor target cells. Significant killing after 105AD7 immunization, which was not related to NK killing, was considered positive.
[e] NK activity was measured by chromium release from K562, NK-sensitive target cells. A significant increase in lytic units of NK activity after 105AD7 immunization was considered positive.
[f] *Mod*, moderately differentiated; ND, not determined.

ing the number of cells required for 50% cytotoxicity as 1 lytic unit. The computer program developed by Pross *et al.* (7) was used to make these calculations. Statistical significance was determined by the Student *t* test.

Results and Discussion

Six patients with primary rectal cancer were skin test negative; they were then immunized with human monoclonal antiidiotype antibody, 105AD7, at tumor diagnosis and at 3 and 6 months postsurgical resection. Each immunization consisted of a skin test of 10 μg of 105AD7 and 48 h later an i.m. dose of 105AD7 (100 μg) on aluminum hydroxide gel, similar to the previous clinical trial in advanced colorectal cancer patients (4).

The kinetics of induction of autologous tumor killing and of enhanced NK activity was carefully studied. Significant killing of autologous tumor cells, which was not due to NK activity, was seen with peripheral blood mononuclear cells or lymph node cells of three patients following immunization with 105AD7 but was not seen on pretreatment biopsies (Fig. 1). Enhanced NK activity postimmunization was seen in 3 of 6 patients (Fig. 2). Pretreatment biopsy target tumor cells from patient PT04 were not killed by either the patients or

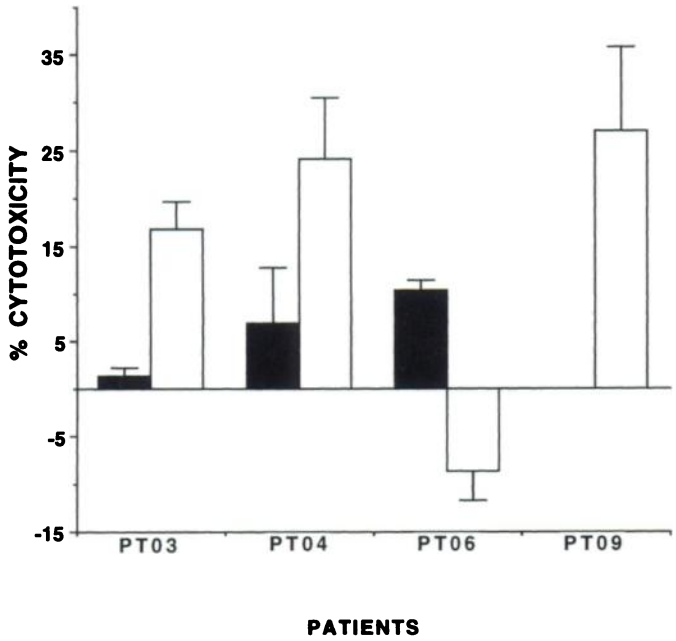

Fig. 1. Cytotoxicity in patients pre- (■) and postimmunization (□) with the human monoclonal antiidiotypic antibody 105AD7 as assayed by chromium release in freshly disaggregated autologous tumor cells. *Columns*, mean; *bars*, SD.

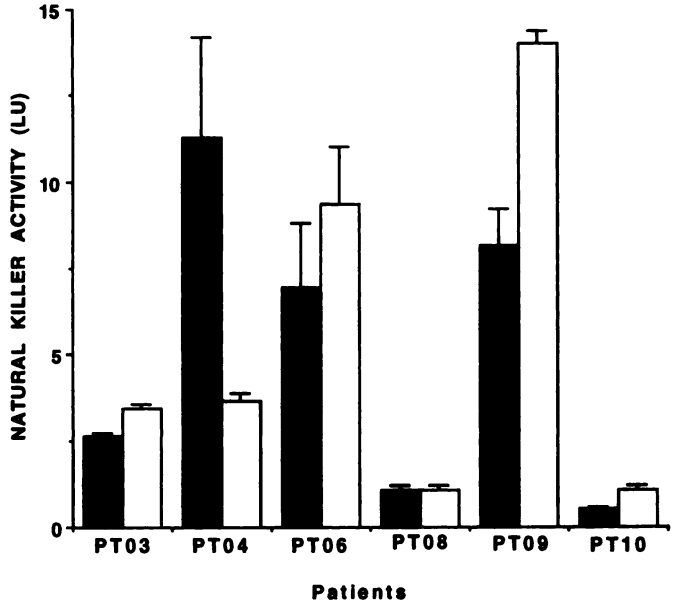

Fig. 2. Cytotoxicity in patients pre- (■) and postimmunization (□) with the human monoclonal antiidiotypic antibody 105AD7 as assayed by chromium release from K562, NK-sensitive target cells. *Columns*, mean; *bars*, SD.

by normal PBMC, although both were active against NK-sensitive target K562, suggesting that PT04 tumor was NK insensitive. The tumor of this patient was resected 6 weeks after immunization, allowing 5 cryopreserved PBMC, fresh PBMC, and lymph node cells from the operation to be tested. PBMC taken 2 and 3 weeks after immunization showed significant lysis of autologous target cells, although the NK activity of these samples, which had been defrosted and cultured in the presence of autologous tumor cells and IL-2 prior to the assay, was very low. No increase in NK activity was seen after 105AD7 immunization in patient PT04 (Fig. 2). In contrast, the pretreatment biopsies of target tumor cells of patient PT09 and of K562 tumor cells were killed by both the patients and normal PBMC, implying that this tumor was NK sensitive and therefore making it impossible to identify T-cell killing. However, significant killing of autologous tumor cells, which was not due to NK activity, was seen in the lymph node cells from patient PT09 postimmunization (Fig. 3). There was also a significant increase in the NK lytic activity in this patient 2 weeks postimmunization (Fig. 2).

Although no significant killing of autologous tumor cells by patient PT03 or by normal PBMC was seen prior to immunization, which would have suggested that PT03 tumor was NK insensitive, both

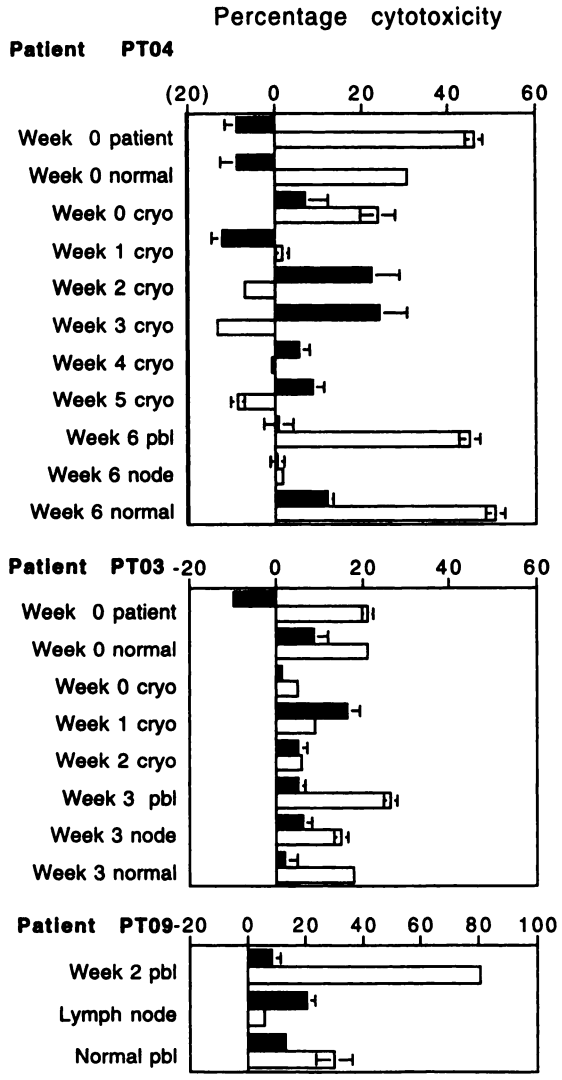

Fig. 3. The kinetics of induction of cytotoxicity in patients PT04, PT03, and PT09 postimmunization with the human monoclonal antiidiotypic antibody 105AD7 as assayed by chromium release in autologous tumor cells (■) and the natural killer sensitive target K562 (□). *Cryo*, lymphocytes which were cryopreserved, defrosted, and then cultured for 3 days in the presence of defrosted and irradiated autologous biopsy extracted tumor cells and IL-2; *normal*, healthy MHC-mismatched donor whose lymphocytes were used to screen the tumors for NK sensitivity. At tumor resection, lymphocytes separated from peripheral blood (*pbl*) or tumor draining lymph nodes (*node*) were both tested. *Columns*, mean; *bars*, SD. Error bars not shown were too small for discrimination.

patient and normal PBMC were active at killing K562 target cells. The tumor was resected 3 weeks after immunization, and significant autologous tumor killing was seen at week 1 in cryopreserved lymphocytes (Fig. 3). Both patients PT03 and PT10 showed killing of NK-sensitive target cells which was enhanced following 105AD7 immunization (Fig. 2).

Thus, treatment of rectal cancer patients by injection of the human monoclonal antiidiotypic antibody produced a time-dependent killing of autologous tumor cells in 3 patients, which appears to be unrelated to NK activity. The main cytolytic effector cells of the immune system are CD8 cells; they express cytotoxic T lymphocytes that recognize (via their specific T-cell receptors) antigenic peptides of eight or nine amino acids complexed with MHC class I molecules on the surface of target cells. Other accessory and adhesion molecules as well as cytokines are also necessary (8, 9) for the expression of efficient cytotoxicity. It is unclear at present whether the killing of autologous tumor cells reported here is a result of clonal expansion of specific cytotoxic T-cells but in this context, our recent DNA- sequencing data have identified several potential MHC class I T-cell epitopes within the CDR regions of the 105AD7 monoclonal antibody.[4] There was no obvious correlation between autologous tumor cell killing and MHC phenotype (Table 1), although the number of patients is too small for any definite conclusions. Analysis of T-cell receptor V-α/V-β gene usage of lymphocytes following 105AD7 immunization is currently being studied to determine if the response is clonal in origin. Due to the difficulties in cell yields it was not possible to block cytotoxicity with nonpolymorphic MHC class I and II monoclonal antibodies. Further studies on T-cell lines derived from immunized patients will address these questions.

MHC class I molecules bind fragments of endogenously synthesized proteins and transport them to the cell surface. In this manner, cells display to the immune system a sampling of their endogenous proteins including viral peptide if a cell is infected (10–12). The peptides presented on MHC class I molecules are generated in a nonlysosomal compartment in cells, probably the cytosol (10). Antigens in the extracellular fluid do not gain access into this processing compartment in most cells and hence, *de novo*, CTL immunity is not classically elicited to soluble proteins such as 105AD7 immunogen. However, recently it has been shown that a subset of antigen-presenting cells can process and present antigens that are present in the extracellular fluids with MHC class I molecules (13). Similarly, protein antigens that are incorporated into immunostimulating complexes or that are encapsulated into certain liposomes can stimulate CTL immunity (14–16). The human antiidiotypic monoclonal antibody may also be presented in the context of MHC class I by one of these mechanisms.

The antiidiotypic antibody could be stimulating CD4 helper T-cells which facilitate activation of preexisting but silent antitumor cytotoxic T-cells. Our initial phase I studies showed elevated levels of serum IL-2 associated with 105AD7 immunization (3). Activation of helper T-cells could also explain the enhanced NK activity seen after 105AD7 immunization in 3 of 6 patients. Human NK cells could be direct effectors against tumor cells; together with T-cells they could produce cytokines such as interferon β, tumor necrosis factor α, and granulocyte-macrophage colony stimulating factor, some of which may enhance antitumor activity from other endogenous lytic effectors (*e.g.*, macrophages and neutrophils) or act by a combination of direct and indirect mechanisms. As induction of CTL often requires help, it could be that the antiidiotypic antibody contains both CD4 helper and CD8 CTL epitopes. In this context, it has been shown that linkage of helper and CTL epitopes, in the sense that they have to be presented by the same antigen-presenting cell, is the basis for productive interaction between the two T-cells and that IL-2 and interferon β play a crucial role (17).

This perioperative rectal cancer study showed an increase in autologous tumor killing following 105AD7 immunization. However, due to the limitations on the availability of both blood and tumor tissue from these patients, it was not possible to identify the lytic effector cells. This question will be addressed in future studies where lymphocytes from 105AD7 immunized patients will be screened for MHC-restricted cytotoxicity against HLA-matched gp72 positive tumor cell lines.

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[4] L. G. Durrant, unpublished observations.

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