A Neoadjuvant Clinical Trial in Colorectal Cancer Patients of the Human Anti-Idiotypic Antibody 105AD7, Which Mimics CD55

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ABSTRACT

Thirty-five patients received 105AD7 human anti-idiotypic antibody vaccination prior to surgery for colorectal carcinoma. Patients were immunized before and also received one to two immunizations after surgical resection of their colorectal cancer. The vaccine was well tolerated with no associated toxicity. Lymphocytic infiltration within the resected tumors was quantified by immunohistochemistry and image analysis. Enhanced infiltration of helper T cells (CD4) and natural killer (NK) cells (CD56) were observed in the tumors from immunized patients when compared with tumors from stage, grade, site, age, and sex matched unimmunized patients. NK activity was increased in the blood, peaking 7–10 days post immunization and then dropping rapidly and correlating with NK extravasation within the tumor. Comparison of the amino acid sequences of 105AD7 anti-idiotypic and the antigen it mimics, CD55, has predicted that patients with HLA-DR1, HLA-DR3, and HLA-DR7 haplotypes should show helper T cell responses following 105AD7 vaccination. Eighty-three percent of patients expressing these haplotypes responded to 105AD7, whereas 88% of patients who failed to express these haplotypes were nonresponders. With a median follow-up of 4 years (range, 2.5–6 years) 65% of patients remained disease free. This trial shows that 105AD7 stimulates antitumor inflammatory responses allowing extravasation within tumor deposits of both helper T cells and NK cells. This represents a way of evaluating immune responses in patients both within the blood and at the tumor site. The study confirms that immunization with a human anti-idiotypic antibody results in immune responses in 83% of patients with a permissive haplotype.

INTRODUCTION

Anti-idiotypic antibodies that bind to antitumor antibodies at their antigen combining sites can act as functional mimics of antigen and stimulate antitumor cell immune responses. Many mouse monoclonal anti-idiotypic antibodies have been isolated which mimic human tumor antigens (1). Clinical trials with these anti-idiotypic antibodies have predominantly stimulated antibody responses (2–4), although antigen-specific T cell responses were also observed (5–7). An alternative approach is to use human anti-idiotypic antibodies. They have the advantage that they are generated from cancer patients and are therefore more likely to stimulate human immune effector cells and in particular T cells. Human anti-idiotypic antibodies were produced by EBV transformation of B cells from patients receiving 17-1A mouse monoclonal antibody therapy (8). The human anti-idiotypes were then used to immunize advanced colorectal cancer patients. Both anti-17-1A antibody and T cell responses were seen in 5 of 6 patients (9). The 105AD7 human monoclonal anti-idiotypic antibody was generated by fusion of a heteromyeloma cell line with B cells from a patient whose tumor was being imaged with a mouse monoclonal antibody that recognized 791Tgp72 antigen (10). A low dose (5 µg) without adjuvant 105AD7 can prime both rats and mice for delayed-type hypersensitivity responses to human tumors cells overexpressing 791Tgp72 (11). However, antibody alone (5–100 µg) or antibody precipitated on alum failed to stimulate antibody responses. In contrast 105AD7 (100 µg) in combination with Freund’s adjuvant or Quill A, resulted in antibody responses that recognized both the anti-idiotypic and the target antigen 791Tgp72.

791Tgp72 antigen has recently been purified and NH2-terminal amino acid sequencing showed homology with the complement regulatory protein CD55. The 791Tgp72 gene has now been cloned from tumor cells and shows complete sequence identity with CD55 (12). The protein is overexpressed by tumor cells to protect them from complement, but its high level of expression may also make it a target for T cells. The homology between 105AD7 and CD55 has now been mapped to three CDR loops and three regions of CD55. For an anti-idiotypic antibody to stimulate antigen-specific T cell responses, a peptide must be processed and presented on MHC from both the anti-idiotypic and the antigen that can be recognized by the same T cell. T cell motif analysis of the homologous regions of 105AD7 and CD55 shows that only the CDRH3 of 105AD7 and the homologous region of CD55 have potential MHC binding mo-
tifs. Interestingly, the predicted affinities are higher for the motifs from 105AD7 than CD55 suggesting that the anti-idio-
type would be a more effective immunogen region, which shows homology with the second small consensus repeat domain shown previously (13). T cell clones are currently being used to map T cell epitopes in 105AD7 and CD55.

In a previously reported Phase I trial using 105AD7 pre-
cipitated on alum, immunization of colorectal cancer patients with extensive liver metastases resulted in cellular antitumor cell immune responses in 10 of 13 patients with no associated toxicity and 3 of 13 patients achieving prolonged stabilization of their disease (14). These immune responses, included lymphocyte proliferation to CD55-expressing tumor cells, enhanced IL-2 production (15) and a switch of CD8 cells from the CD45RA (naïve) to CD45RO (memory/activated) phenotype. The CDRH3 of 105AD7 has potential HLA-A1, -3, -24 and HLA-DR1, -3, -7 binding motifs. Patients showing either a CD8RA to RO conversion or autologous tumor cell killing all expressed one of the predicted HLA-A haplotypes. Patients showing in vitro blastogenesis responses to CD55-overexpress-
ting tumor cells or enhanced IL-2 all expressed HLA-DR1, -3, or -7 phenotypes (13). As in the mouse studies, no antibody re-
sponses were generated to low dose antibody precipitated on alum. All of the cellular immune responses were measured in the peripheral blood and the question arose as to whether the immune response ever reached the tumor site. A neoadjuvant trial was therefore designed whereby patients were immunized prior to tumor resection and were then boosted twice at 6 weekly intervals post surgery. Preliminary results on rectal cancer pa-
tients showed evidence for autologous tumor cell killing that was unrelated to NK killing in 3 of 4 patients and direct NK killing in 3 of 6 patients (16). This trial has now recruited 36 patients, and the antitumor cell immune responses and their disease course following 105AD7 immunization have now been assessed and are reported in this study.

MATERIALS AND METHODS

Patients. This trial was run under the auspices of the CRC, United Kingdom, Phase I targeting trial committee. Local ethical approval was obtained from the recruiting hospital. Pa-
tients with histopathologically proven colorectal adenocarcinoma who were scheduled for elective surgery were recruited. Patients had to have a WHO performance status of 0 –2, a hemoglobin of >10 g/dl, a WBC count >2 × 10^9/liter and platelets >50 × 10^9/liter. All patients had normal renal and liver function (no more than 25% deviation from normal values). All patients gave written informed consent and were registered with the CRC data center. Patients with any acute intercurrent illness, autoimmunne or chronic hematological disorders, or receiving other concomitant anticancer therapy were excluded. No women of child-bearing age or having planned preoperative radiother-
apy to primary rectal tumors were included. Patients could receive postoperative chemotherapy if indicated. However, they completed their postoperative 105AD7 course 3 months after the completion.

Human Monoclonal Antibody. Clinical grade human monoclonal antibody was produced as previously described (15) using the guidelines of the CRC (17). 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 skin test doses or as aluminum hydroxide gel (alHydrogel 85, Superphos Biosector, Vebea Denmark) precipitated in i.m. doses of 100 µg antibody/ml. The antibody can be stored at 4°C. Stability studies have shown that the antibody can be stored at 4°C for a minimum of 5 years with no loss in binding activity.

Clinical Protocol. The clinical protocol initially stated that patients should receive an i.d. skin test of 105AD7 and, if after 24 h there was no adverse reaction, they could then receive the i.m. dose of 105AD7 precipitated on alum. As none of the first 27 patients showed a skin test response, permission was received to drop the intradermal dose for the last 9 patients. The first 21 patients were given 100 µg of 105AD7/alum, and the remaining 15 received a 50 µg dose to see if reducing the dose gave a further improvement in the immune response. Patients received an initial dose of 105AD7 at diagnosis of their colore-
rectal cancer and then were boosted at 6 and 12 weeks following their surgical resection. The final 9 patients receiving just the i.m. injections were given two immunizations 1 week apart prior to surgical resection and were then boosted postoperatively at 6 and 12 weeks. Venous blood samples were taken into preservative-free heparin before administration of anti-
idiotypic antibody and then at 7- to 10-day intervals until tumor resection. Blood samples were separated on Lymphoprep (Flow Laboratories, Irvine, Scotland) and peripheral blood mononuclear cells were frozen in liquid nitrogen using DMSO as a cryopreservative.

Immunohistochemistry. Quantitative immunohistochem-
istry was used to measure infiltration of helper T cells (CD4), cytotoxic T cells (CD8), natural killer cells (CD56), and macro-
phages (CD68) within tumors of immunized patients as compared with stage, grade, and site matched unimmunized tumors. Samples were taken immediately following tumor resection by a pathologist from two edges and from the center of the tumor. Tumor tissue from both immunized and unimmunized patients was stored in liquid nitrogen. Tumor blocks from both the edges and center from immunized and a stage, grade, and site matched control tumor selected from the tumor bank were selected. Sections (5 µm) were air-dried for 5 min and then fixed in acetone for 10 min. After air drying overnight they were rehydrated with Tris-buffered saline and 100 µl of 20% rabbit serum was added for 20 min. The slides were then coded by a third party, and the staining and analysis was performed blind. 100 µl of either CD4 (1:40; Becton Dickinson, Cowley, Oxford, United Kingdom) or CD56 (1:40; Becton Dickin-
son) or CD8 (1:20; gift from Dr. A. King, Aberdeen University, Scotland) or CD68 (1:40; Dako, High Wycombe, United King-
dom) or normal mouse immunoglobulin (5 µg/ml; Sigma) was added to consecutive sections from each region of each tumor. After 1 h the slides were washed and 100 µl of biotinylated rabbit anti-mouse (Sigma) diluted in Tris-buffered saline containing 4% human serum was added for 30 min. Following a further wash 100 µl of Vectastain ABC reagent (Vector Laboratories, Peterborough, United Kingdom) was added for 30 min. Staining was developed

The abbreviations used are: IL-2, interleukin 2; NK, natural killer; i.d., intradermal; CRC, Cancer Research Campaign.
A Neoadjuvant Trial of a Human Anti-Idiotype

Briefly cryopreserved lymphocytes were defrosted and incubated for 4 h at 37°C and then tested for cytotoxicity against K562 cells as targets. Varying numbers of effector cells were added to 10^4 chromium labeled (10^6 cells labeled with 100 μCi of [51Cr]chromium for 45 min at 37°C) target cells to produce ratios of 50:1–12.5:1. Chromium release was measured in 100 μl of supernatant at 4 h. The percentages of chromium released and cytotoxicity were calculated, and the relationship between the percentages of cytotoxicity and effector cell numbers was fitted using an exponential equation. Lytic units per 10^6 effector cells were calculated, defining the number of cells required for 10% cytotoxicity as 1 lytic unit. The computer program developed by Pross et al. (20) was used to make these calculations. Statistical significance was determined by Student’s t test.

RESULTS

Thirty-five patients were recruited prospectively from a surgical clinic (Table 1). The group consisted of 24 men and 11 women with a mean age of 71.3 years (range, 56–87). Of these, 69% had rectal tumors and 31% had colonic tumors, and 86% of women with a mean age of 71.3 years (range, 56 – 87) were added to 10^4 chromium labeled (10^6 cells labeled with 100 μCi of [51Cr]chromium for 45 min at 37°C) target cells to produce ratios of 50:1–12.5:1. Chromium release was measured in 100 μl of supernatant at 4 h. The percentages of chromium released and cytotoxicity were calculated, and the relationship between the percentages of cytotoxicity and effector cell numbers was fitted using an exponential equation. Lytic units per 10^6 effector cells were calculated, defining the number of cells required for 10% cytotoxicity as 1 lytic unit. The computer program developed by Pross et al. (20) was used to make these calculations. Statistical significance was determined by Student’s t test.

**Table 1** Patient demographics and immunization schedules

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Dose</th>
<th>Immunization (weeks)</th>
<th>Operation (weeks)</th>
<th>Tumor site</th>
<th>Duke’s stage</th>
<th>Other therapy</th>
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<td>1 (1992)</td>
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<td>0, 16, 40</td>
<td>3</td>
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</tr>
<tr>
<td>3</td>
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<td>0, 14, 38</td>
<td>3</td>
<td>Rectum C</td>
<td>No</td>
<td></td>
</tr>
<tr>
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<td></td>
</tr>
<tr>
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<td>0, 13, 37</td>
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<td></td>
</tr>
<tr>
<td>6</td>
<td>10 i.d. + 100 i.m.</td>
<td>0, 8, 14</td>
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<td></td>
</tr>
<tr>
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<td>0, 12</td>
<td>8</td>
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<td></td>
</tr>
<tr>
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<td>0, 7, 14</td>
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<td>Rectum B</td>
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<td></td>
</tr>
<tr>
<td>9</td>
<td>10 i.d. + 100 i.m.</td>
<td>0, 7</td>
<td>4</td>
<td>Rectum C</td>
<td>No</td>
<td></td>
</tr>
<tr>
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<td>0, 9, 15</td>
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<td>Sigmoid C</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>10 i.d. + 100 i.m.</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
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<td>2</td>
<td>Ascc C</td>
<td>No</td>
<td></td>
</tr>
<tr>
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<td>Ascc B</td>
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<td></td>
</tr>
<tr>
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</tr>
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<td></td>
</tr>
<tr>
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<td>5FU/FA × 6</td>
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</tr>
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<td>0, 16</td>
<td>4</td>
<td>Ascc B</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>18</td>
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<td>0, 10, 17</td>
<td>4</td>
<td>Rectum C</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>10 i.d. + 100 i.m.</td>
<td>0, 8, 15</td>
<td>2</td>
<td>Sigmoid A</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>10 i.d. + 100 i.m.</td>
<td>0, 1</td>
<td>Rectum C</td>
<td>5FU/FA × 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21 (1995)</td>
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<td>3</td>
<td>3</td>
<td>Rectum C</td>
<td>5FU/FA × 6</td>
<td></td>
</tr>
<tr>
<td>22</td>
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<td>2</td>
<td>Sigmoid B</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
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<td>0, 8</td>
<td>1</td>
<td>Ceacal C</td>
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<td></td>
</tr>
<tr>
<td>24</td>
<td>10 i.d. + 50 i.m.</td>
<td>0, 8, 16</td>
<td>1</td>
<td>Sigmoid D</td>
<td>RTH</td>
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</tr>
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<td>25</td>
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<td>0, 9, 15</td>
<td>3</td>
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<td></td>
</tr>
<tr>
<td>26</td>
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<td>1</td>
<td>Rectum A</td>
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<td></td>
</tr>
<tr>
<td>27</td>
<td>50 i.m.</td>
<td>0, 1, 8, 17</td>
<td>1</td>
<td>Rectum B</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>50 i.m.</td>
<td>0, 8</td>
<td>3</td>
<td>Rectum C</td>
<td>5FU/FA × 6</td>
<td></td>
</tr>
<tr>
<td>29 (1996)</td>
<td>50 i.m.</td>
<td>0, 1, 9, 17</td>
<td>2</td>
<td>Sigmoid A</td>
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<td></td>
</tr>
<tr>
<td>30</td>
<td>50 i.m.</td>
<td>0, 1, 10</td>
<td>2</td>
<td>Rectum A</td>
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</tr>
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<td>31</td>
<td>50 i.m.</td>
<td>0, 1, 41</td>
<td>1</td>
<td>Rectum C</td>
<td>5FU/FA × 6</td>
<td></td>
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<td>32</td>
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<td>2</td>
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<td>33</td>
<td>50 i.m.</td>
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<td>0, 1</td>
<td>2</td>
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</table>

 Patients’ number and year of entry.

 Patients received 10 μg of 105AD7 alone by i.d. injection and either 100 or 50 μg of 105AD7 precipitated on alum by i.m. injection.

 Time in weeks between first immunization with 105AD7 and tumor resection.

 Tumor sites defined as rectum, sigmoid colon, ascending colon (Asc), or Ceacal colon (Ceacal).

 Modified Duke’s stage with D describing patients with liver metastases.

 Other therapy postsurgical resection of the tumor. 5FU/FA × 6, six weekly cycles of 5-fluorouracil and folinic acid; RTH, radiotherapy.

NK Activity. NK activity was measured as previously described (19). Briefly cryopreserved lymphocytes were defrosted

Image Analysis. Sections were viewed under ×125 magnification, and the image was digitized and transferred by camera to an Apple Macintosh Quadra 660AV computer. Using the NIH image program it was possible to quantify the degree of staining and express it as a pixel count. Sections were analyzed from two edges and the center of the tumor. Infiltration was quantified on five randomly selected areas for each section. A cumulative pixel count of all 15 areas (three tumor areas, five fields per area) for each antibody for each tumor was computed. Immunized and unimmunized tumors were coded prior to staining and analysis to ensure that there was no observer bias. Cumulative pixel counts for each antibody on immunized and matched unimmunized tumor was decoded and analyzed for significance by a Wilcoxon paired signed rank test.

NK Activity. NK activity was measured as previously described (19). Briefly cryopreserved lymphocytes were defrosted

and incubated for 4 h at 37°C and then tested for cytotoxicity against K562 cells as targets. Varying numbers of effector cells were added to 10^4 chromium labeled (10^6 cells labeled with 100 μCi of [51Cr]chromium for 45 min at 37°C) target cells to produce ratios of 50:1–12.5:1. Chromium release was measured in 100 μl of supernatant at 4 h. The percentages of chromium released and cytotoxicity were calculated, and the relationship between the percentages of cytotoxicity and effector cell numbers was fitted using an exponential equation. Lytic units per 10^6 effector cells were calculated, defining the number of cells required for 10% cytotoxicity as 1 lytic unit. The computer program developed by Pross et al. (20) was used to make these calculations. Statistical significance was determined by Student’s t test.

RESULTS

Thirty-five patients were recruited prospectively from a surgical clinic (Table 1). The group consisted of 24 men and 11 women with a mean age of 71.3 years (range, 56 – 87). Of these, 69% had rectal tumors and 31% had colonic tumors, and 86% of
The tumors were classified on routine histopathology as being moderately differentiated. Using the modified Duke’s staging, which ascribes D to patients with liver metastases, 9 patients had stage A tumors, 10 stage B, 14 stage C, and 2 stage D. Four patients received adjuvant chemotherapy and one patient received postoperative radiotherapy. The vaccine was well tolerated with no associated toxicity. Compliance was good with 21 of 35 patients receiving preoperative immunizations and 2 postoperative boosts as indicated by the protocol. Six patients failed to return postoperatively, and eight patients received only one postoperative dose. The median time between first immunization and tumor resection was 3 weeks (range, 1–8 weeks).

Of the 35 patients recruited, there was sufficient tumor tissue from 22 patients to allow detailed sampling while leaving sufficient material for histopathological evaluation. For each immunized tumor a control tumor matched for site, stage, grade, and patient age and sex was selected from a tissue bank of over 300 cryopreserved tumor specimens sampled and stored by the same pathologist. Three areas of the tumor were sampled and five separate fields/antibody/section were quantified using image analysis. Fig. 1 shows the cumulative pixel count for each tumor and its paired control tumor stained for CD4, CD56, CD8, CD68 and with normal mouse immunoglobulin as a control. Results were analyzed for signif-
significance using a paired Wilcoxon ranking test. There was a significant infiltration of CD4 ($P = 0.043$) and CD56 ($P = 0.041$) cells, whereas there was no significant difference in CD8 infiltration between immunized and control tumors. Staining with irrelevant mouse immunoglobulin was consistently low, and there was no difference between immunized and control tumors confirming that the staining for infiltrating cells was specific. The level of infiltration of CD68 was similar in the majority of control and immunized tumors with only two tumors showing an increase and two showing a decrease in immunized as compared with control tumors. This validates the blind pairing and staining of immunized tumors with control tumors from a large tissue bank and agrees with the statistical analysis that suggested that the increase in CD4 and CD56 cells within immunized tumors was not a random event.

Fig. 2 illustrates the variation in infiltration of the different immune cells within individual immunized and paired control tumors. The results of six patients with enhanced CD4 and/or CD56 infiltration within immunized tumors compared with control tumors are shown. Fig. 3 illustrates the infiltration of immune cells in six patients where there was no enhanced infiltration of CD4 or CD56 in immunized compared with control tumors. If the infiltration of any cell type was 25% greater in the immunized compared with the control tumor then the patient was defined as a responder to 105AD7 vaccine (Table 2).

Thirteen of the 22 patients analyzed showed enhanced infiltration of CD4, four tumors had a 25–100% increase, three patients had a 100–200% increase, and six had an increase of greater than 200% compared with matched control tumors (Table 2). Tumors in the remaining nine patients showed similar levels of CD4 infiltration to matched control tumors with five showing a less than 25% decrease and four a 25–100% decrease. Control tumors showed low levels of tissue NK cells (Fig. 1) but 12 of the immunized tumors showed enhanced infiltration. One tumor had a 25–100% increase, three patients had a 100–200% increase, and eight had an increase of greater than 200% compared with matched control tumors. Tumors in the remaining eight patients showed similar levels of CD56 infiltration to matched control tumors, with five showing a less than 25% decrease and three a 25–100% decrease. Of the 13 tumors showing enhanced CD8 infiltration, five showed a 25–100% increase, two a 100–200% increase, and six an increase greater than 200%. However, six tumors showed a decrease in CD8 infiltration between immunized and control patients with two showing a 100–200% decrease and four showing greater than 200% decrease. The remaining three patients showed similar levels of infiltration between control and immunized tumors. In summary, 16 patients showed an infiltration of CD4, CD56, or both, and in 12 of these patients there was also enhanced infiltration of CD8 but not CD68 cells. The remaining six patients showed no significant infiltration of CD4 or CD56 cells.
samples were obtained prior to tumor resection. Fig. 5 shows the ever, in seven patients two or more postimmunization blood sample was obtained prior to surgery. How-

ever, the NK activity in both patients was high prior to immu-

nation.

Kinetics of the NK responses in the four patients who showed an NK response. The kinetics were extremely inter-

esting with responses peaking at 5–10 days and then showing a rapid decline. There was a good correlation between enhanced

NK infiltration and prior to surgery. Fourteen of the 23 patients showed significant increases in NK activity 7–10 days

before immunization and prior to surgery. The remaining three patients did not show an NK response. The kinetics were extremely interesting with responses peaking at 5–10 days and then showing a rapid decline. There was a good correlation between enhanced NK infiltration and enhanced NK activity in 8 of 10 patients (Table 2). Two patients showed enhanced infiltration of CD4 and CD56 cells but failed to show enhanced NK activity. However, the NK activity in both patients was high prior to immunization.

Previous studies suggested an association between MHC
phenotype and immune responses in patients. Blood was available from 21 patients for HLA-DR typing. There was a good correlation between HLA-DR phenotype and CD4/CD56 infiltration and NK activity with 17 of 20 patients responding as predicted (Table 2). Twelve patients expressed HLA-DR1, -3, -7 and -10 of these patients showed an antitumor cell immune response. Eight patients failed to express any of these haplotypes, and seven of them failed to respond in any assay.

Whether patients received an intradermal dose of 105AD7 did not appear to affect the ability of 105AD7 to induce immune responses because 18 (69%) of 26 patients receiving an intradermal dose responded compared with 7 (77%) of 9 receiving only the i.m. dose. The i.m. dose did not appear to affect the immune response [100 μg: 15 (75%) of 20; 50 μg: 10 (66%) of 15].

One patient died due to postoperative complications. Of the remaining 34 patients, 22 remain disease free with a minimum follow-up of 2.5 years (median 4 years). This gives an overall survival of 65% that compares favorably with a 4-year survival of 40% for colorectal cancer. However, survival is highly dependent upon Duke’s stage with Duke’s A patients having an expected 90% and Duke’s D a 5% 5-year survival. Table 3 shows the survival for the vaccine-treated patients for each Duke’s stage. The numbers are too small for statistical evaluation, but of interest are the Duke’s C patients, with 9 (64%) of 14 of them remaining relapse-free (median follow-up, 4.5 years). Of the nine Duke’s C patients who survived, eight of nine responded to 105AD7, whereas of the five patients who died, only one had responded to 105AD7 vaccination.

DISCUSSION

Previous studies with the human anti-idiotypic antibody 105AD7 have shown that this vaccine can stimulate both helper and cytotoxic T cell responses in the peripheral blood of immunized colorectal cancer patients (14). In this study we have extended these observations to immune responses within tumors of immunized patients. 105AD7 stimulated tumor infiltration of CD4 cells. Previous studies have shown that these CD4 cells are activated and express the CD25 (IL-2 receptor). Activated CD4 cells can extravasate at the site of the tumor, and upon stimulation by peptide presented on MHC class II molecules, can release cytokine cytokines and send out inflammatory signals that aid in the recruitment of nonspecific cells such as natural killer cells and macrophages. The enhanced infiltration of CD4 cells within tumors following 105AD7 immunization suggests but does not prove that these cells recognize processed antigen at the tumor site. Previous studies have shown that 105AD7 can stimulate delayed-type hypersensitivity responses in mice to human tumor cells expressing 791Tgp72 but not antigen-negative tumors. Similarly 5-day in vitro blastogenesis responses were specific to tumor cells expressing 791Tgp72. Because 791Tgp72 has now been identified as CD55, recombinant protein can be produced in sufficient quantities to allow antigen-specific responses to be studied. 105AD7-specific CD4 T cell clones can be challenged with recombinant CD55.
Natural killer cells do not usually extravasate within tissues but circulate in the blood. In response to CD4-mediated inflammatory signals, NK cells can extravasate within the tumor. The kinetics of NK activity in the blood showed a rapid response followed by a rapid decline. This may indicate that the NK cells are only transiently activated or it may relate to their extravasation from the blood to the tumor site as indicated by quantitative immunohistochemistry. NK cells kill tumor cells that have lost MHC expression. Earlier studies have shown that 70% of colorectal tumors have some loss of HIC class I antigens (21). These results suggest that the immune responses seen previously in the blood of 105AD7-immunized patients can reach the tumor site. It is now important to assess if these immune responses have any antitumor effects. Because CD4 cells releasing cytotoxic cytokines and NK cells usually kill by apoptosis, it may be possible to measure tumor cell apoptosis on the resected specimen following neoadjuvant immunization. Preliminary results of a new prospective study suggest that 105AD7 does indeed induce significant apoptosis in immunized compared with control tumors (unpublished observation).

Although there is only limited data available on the dosing and timing of 105AD7 administration, the dose of 105AD7 injected did not appear to be critical, because there was no obvious decrease in the number of responders when it was reduced from 100 to 50 μg. Our previous studies have shown that increasing the dose of 105AD7 to 200 μg resulted in a reduced number of patients producing a cellular immune response. This is also in line with the animal studies in which a higher dose and a more potent immune adjuvant than alum induced Th2 rather than a cellular immune response. The omission of the intradermal dose did not have any effect on the number of responders, suggesting that it was the i.m. dose that was stimulating the immune response.

It may be important to increase the number of immunizations because it is difficult to generate a sustained memory response against a self antigen (22). Patients entered into this trial were offered one to two immunizations prior to surgery and one to two postoperative boosts 6 weeks apart. New patients are now boosted postoperatively every 3 months for the 2-year period when colorectal cancer is most likely to recur. Considering the limited time scale of vaccination, it was therefore of interest that 63% of patients remain disease free 2.5–6 years following immunization. The Duke’s C patients did particularly well with only 5 of 14 deaths. Eight (89%) of the nine Duke’s C patients who responded to 105AD7 also survived. Furthermore, a more appropriate choice of immune adjuvant may further enhance the immune response. Granulocyte macrophage colony-stimulating factor has been shown to enhance cellular immune responses in cancer patients (23).

Quantifying inflammatory infiltration within tumors suffers from the problem of large intra- and intertumor variation. In this study this was minimized by carefully matching tumors for stage, grade, and site and also selecting tumors from patients of the same gender and age. However, some variability still exists. This was a particular problem when quantifying CD8 infiltrates: Although enhanced infiltration of CD8 cells was observed in the 12 patients showing enhanced CD4 infiltration, the levels of CD8 within tumors was in general more variable with some unimmunized tumors showing unexpectedly high levels of CD8 infiltration. There was no significant enhancement of CD8 infiltration in immunized as compared with control tumors. It may be necessary to study activation of CD8 cells, because studies on the peripheral blood of 105AD7-immunized patients showed a switch from CD8 RA (naïve) to CD8RO (activated) phenotype but no difference in the numbers of CD8 cells (13). Recent studies suggest that a better approach may be to disaggregate tumors and then stain the infiltrating leukocytes for either intracellular cytokines (24) or with tetramers (25).

Molecular analysis of the anti-idiotype mimicry of CD55 and 105AD7 has shown that the anti-idiotype shows amino acid homology between CDR1, H2, H3, and 3 regions of CD55. These regions have all been synthesized as peptides and been shown to be the binding site for the Ab1 791T/36 that recognizes both CD55 antigen and 105AD7 anti-idiotype (Spendlove et al., unpublished observations). T cell motif analysis predicted that 105AD7 should induce helper T cell responses in patients with HLA-DR1, -3, and -7 haplotypes. Earlier studies in 105AD7-immunized patients, where in vitro proliferation of T cells to CD55-expressing tumor cells was measured, confirmed that patients with the predicted haplotypes responded. This study extended these observations to a further group of 21 patients by showing that enhanced CD4/CD56 infiltration in immunized tumors and enhanced NK activity was induced in 83% of patients with HLA/DR1, -3, and -7 phenotypes and no responses in 88% of patients with other haplotypes.

In conclusion, the human anti-idiotype 105AD7 mimics distinct regions of the CD55 antigen and stimulates helper T cells and NK cells. These helper T cells infiltrate tumors allowing extravasation of the activated NK cells. 83% of patients expressing the permissive HLA-DR1, -3, and -7 haplotypes show antitumor responses and 65% of patients survived 2.5–6 years following immunization. These results encourage further clinical evaluation of this approach with a stronger adjuvant and a more aggressive immunization protocol.

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