Clinical significance of changes of perioperative T cell and expression of its activated antigen in colorectal cancer patients

WANG Yi-Xing, RUAN Can-Ping, LI Li, SHI Jing-Hua and KONG Xian-Tao

Subject headings: colorectal neoplasms; perioperative period; T lymphocytes; surface antigen

INTRODUCTION

Immune function status is corresponded to genesis and progress of tumor, and cell immune plays a major role in anti-tumor immunity. In recent years, there has been more and more interest in the effect of tumor on functional imbalance of T lymphocyte subgroups. In order to study changes of immune status in colorectal cancer patients before and after operation, we determined six kinds of T lymphocyte surface antigens including CD3, CD4, CD8, CD16, CD69 and CD3+/HLA-DR+ in 35 patients with colorectal cancer from April 1996 to March 1997, and the results were compared with those in patients with benign diseases.

MATERIAL AND METHODS

Patients

Among the twenty-five patients with benign disease in the control group, sixteen were patients with inguinal hernia, nine with varicose, their average age was 46.61 ± 11.63. They did not take any medicine recently and had no complications. Among the thirty-five patients in the colorectal group, eighteen were male and seventeen female. Their average age was 60.13 ± 8.15. All of them were operated upon, twenty-seven patients received radical resection and eight patients palliative resection. The diagnosis was confirmed by pathological examination.

Material

A murine monoclonal antibody to human CD3, CD4, CD8, CD16, CD69 and a double-color fluorescent labeled CD3-FITC/HLA-DR-PE were purchased from Coulter-Immunotech Company.

Methods

Peripheral vein blood was collected in heparin tubes 3 days before operation in control, 3 days before operation and 10 days after operation in cancer patients respectively. Ten µL fluorescent labeled antibodies (Coulter) was mixed into 100 µL heparinized blood (or 10^6 cells with nucleus) in a tube. The mixture was incubated at room temperature for 15 min, and was processed in a Q-prep machine and determined in a Coulter Epics XL flow cytometer (Coulter Company, USA) with relative softwares. The Dot Plot was made according to the detected value of the forward scatter light and the side scatter light (at a 90° angle from the laser axis) of flowing cells excited by an air-cooled 488 nm argon-ion laser. Green and red emissions of the lymphocyte group were detected with a 530 nm ± 10 nm bandpass filter and a 515 nm ± 10 nm bandpass filter respectively. The same type of non-fluorescent-labeled murine monoclonal antibodies was used as control in each group of samples. A combination of volt of the photomultiplier tubes (PMT1 and PMT2) was adjusted according to the non-fluorescent-labeled murine monoclonal antibodies to keep background fluorescent below 2% and CD3/CD4 color complement was adjusted in permitting width.

Statistical analysis

Data was analyzed by Student’s t test and Chi-square test.

RESULTS

Changes of T cell and its subgroups in colorectal cancer patients before and after operation

The results in Table 1 show that: (1) CD3, CD4 and CD8/CD4 were significantly lower in cancer patients before operation than those in control while CD8 was much higher in cancer patients (P < 0.05). (2) T cell and its subgroups changed obviously after operation. CD8 increased obviously in cancer patients after operation than those before operation (P < 0.05), but CD4 decreased obviously (P < 0.05). No significant difference was found in CD3, CD4 and CD8/CD4 of the postoperative cancer patients and the controls.
CD16 and CD69 T cell operation were lower than those in control, but CD8 was growing [1,2]. No statistical difference was found in serum immune suppressive factor along with the tumor activated T cell CD3+/HLA-DR+ between the cancer patients.

Both T cell subgroups and T cell activated antigen changed obviously in colorectal cancer patients after operation with CD3, CD4/CD8, CD16 and CD69 increased significantly, and CD8 decreased obviously. Except the activated T cell CD3+/HLA-DR+ which was much higher than that in control, no significant difference was found in other parameters between the colorectal cancer patients after operation and the controls. The mechanism is that immune suppression in colorectal cancer patient is caused by the soluble immune suppression factor originated from tumors related to differentiation. Since the level of the immune suppression factor decreased, the body immune function and host antitumor immune function recovered gradually after the tumor was resected, strong T cell immune response induced by MHC II antigen HLA-DR activated CD2 positive T cell to produce cellular factors, and augmented effect of CD2 or CD4 positive T cell mediated ADCC[3-5]. Increased CD2 positive T cell, T help cell and CD16 expression, and decreased suppressive T lymphocyte after operation showed that resection of tumor is helpful in improving patient cell immune functions. Increased T cell activated antigen CD69 and CD3+/HLA-DR+ showed that host cell immune function was enhanced. So we hold that radical or palliative excision of colorectal tumor may be helpful in enhancing patient immune functions and postoperative treatment. Monitoring patient immune status after operation may have definite clinical significance in predicting the prognosis of patients.

REFERENCES

2 Ortado JR, Manson A, Overton R. Lymphokine-activated killer phenomenon: relative contribution of peripheral blood natural killer cells and T lymphocytes to cytolysis. J EXP Med, 1986;164:1193
3 Bi AH. Medical immunology. The People's Surgeon Press, Beijing, 1996:106
4 Jackson PA, Green MA, Marks CG, King RJB, Hubbard R, Cook MG. Lymphocyte subset infiltration patterns and HLA antigen status in colorectal carcinomas and adenomas. Gut, 1996;38:85

Expression of T cell activated antigen and CD16 in patients with colorectal cancer

The results in Table 2 show that: (1) CD16 and CD69 were significantly lower in cancer patients before operation than those in control, and no significant difference was found in CD3+/HLA-DR+ between the postoperative cancer patients and the control. (2) T cell activated antigen CD69, CD3+/HLA-DR+ and CD16 increased obviously postoperatively (P<0.05). No significant difference was found in CD16 and CD69 between the postoperative cancer patients and the controls, while CD3+/HLA-DR+ was much higher than that in control (P<0.01).

### DISCUSSION

It was found in the study that CD3, CD4, CD4/CD8, CD69 and CD16 on NK cell surface in cancer patients before operation were lower than those in control, but CD8 was much higher than that in control. The cell immune function decreased significantly in colorectal cancer patients before operation. Elevation of CD8 was caused by increase of T suppressive cells (Ts), while reduced CD16 was due to decrease of NK cells and increase of serum immune suppressive factor along with the tumor growing[1,2]. No statistical difference was found in activated T cell CD3+/HLA-DR+ between the cancer patients before operation and the controls. It might be caused by the on-going of TH cell mediated ADCC effect when TH cell (CD4) having recognized APC antigen in body with tumor was activated. Though the body immune function was suppressed at different extent, activation of T cell which was important in cell immune was still going on[3].

Both T cell subgroups and T cell activated antigen changed obviously in colorectal cancer patients after operation with CD3, CD4/CD8, CD16 and CD69 increased significantly, and CD8 decreased obviously. Except the activated T cell CD3+/HLA-DR+ which was much higher than that in control, no significant difference was found in other parameters between the colorectal cancer patients after operation and the controls. The mechanism is that immune suppression in colorectal cancer patient is caused by the soluble immune suppression factor originated from tumors related to differentiation. Since the level of the immune suppression factor decreased, the body immune function and host antitumor immune function recovered gradually after the tumor was resected, strong T cell immune response induced by MHC II antigen HLA-DR activated CD2 positive T cell to produce cellular factors, and augmented effect of CD2 or CD4 positive T cell mediated ADCC[3-5]. Increased CD2 positive T cell, T help cell and CD16 expression, and decreased suppressive T lymphocyte after operation showed that resection of tumor is helpful in improving patient cell immune functions. Increased T cell activated antigen CD69 and CD3+/HLA-DR+ showed that host cell immune function was enhanced. So we hold that radical or palliative excision of colorectal tumor may be helpful in enhancing patient immune functions and postoperative treatment. Monitoring patient immune status after operation may have definite clinical significance in predicting the prognosis of patients.

### Table 1  Change of T cell and its subgroups in colorectal cancer patients before and after operation (%,

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cases</th>
<th>CD3</th>
<th>CD4</th>
<th>CD8</th>
<th>CD4/CD8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>25</td>
<td>68.41</td>
<td>39.88</td>
<td>25.15</td>
<td>1.79</td>
</tr>
<tr>
<td>Colorectal cancer before operation</td>
<td>35</td>
<td>62.57</td>
<td>38.81</td>
<td>30.71</td>
<td>1.13</td>
</tr>
<tr>
<td>Colorectal cancer after operation</td>
<td>35</td>
<td>65.38</td>
<td>37.31</td>
<td>25.26</td>
<td>1.69</td>
</tr>
</tbody>
</table>

P<0.05 vs control and colorectal cancer after operation.

### Table 2  Expression of T cell activated antigen and CD16 in patients with colorectal cancer (%,

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cases</th>
<th>CD16</th>
<th>CD4</th>
<th>CD8</th>
<th>CD4/CD8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>25</td>
<td>11.11</td>
<td>10.98</td>
<td>4.44</td>
<td>4.44</td>
</tr>
<tr>
<td>Colorectal cancer before operation</td>
<td>35</td>
<td>7.37</td>
<td>5.99</td>
<td>2.07</td>
<td>3.81</td>
</tr>
<tr>
<td>Colorectal cancer after operation</td>
<td>35</td>
<td>11.62</td>
<td>10.87</td>
<td>7.62</td>
<td>7.62</td>
</tr>
</tbody>
</table>

P<0.05 vs control; P<0.01; P<0.05 vs colorectal cancer after operation.