IMMUNOGENETIC STUDIES OF FAMILIAL LARGE BOWEL CANCER

Mitsuo Katano, Hiroshi Fujiwara, Kiyokazu Toyoda, and Motomichi Torisu

Division of Clinical Immunology, Department of First Surgery, Kyushu University School of Medicine*

We have recently found a familial series of colon cancers without polyposis. The characteristics of the family K are as follows. (1) Primary colon cancer affects three generations (five of 25 relatives had colon cancer). (2) The age of onset of cancer is about 20 to 30 years earlier than would be expected among the general population (ages ranging from 16 to 49 years). (3) All of the patients are male. (4) The proband was a 16-year-old boy, one of identical twins. The other twin shows no symptoms at present.

A total of 17 relatives in the family K was tested for lymphocyte blast transformation (PHA responsiveness). One of two affected members with colon cancer, one rectal polyp patient and 4 of 14 cancer-free relatives (29%) in the family K showed significantly decreased phytohemagglutinin (PHA) responsiveness in vitro ($P<0.0025$). When the age is limited to relatives over 20 years, which seems to be the high risk age for developing cancer in the family K, 5 of 7 relatives (71%) showed significantly decreased PHA responsiveness ($P<0.001$).

This study clarified the HLA type in 21 relatives, including three relatives who died before this test, retrospectively. Nine relatives had A9-Bw35 haplotype and four of them (44%) had a current or previous colon cancer (relative risk = 8.8). As for those over the age of 20, five persons had A9-Bw35 and four of these (80%) developed colon cancer. On the other hand, four relatives over 20 years of age did not have the A9-Bw35 haplotype and none of these had developed colon cancer. These observations suggested that immunogenetic factors might be involved in the development of colon cancer in members of the family K.

Key words: Colon cancer — Lymphocyte blast formation test — HLA type — Familial polyposis of colon — Immunogenetic study

Several reports demonstrate that colon cancer aggregates in families who show no evidence of a precancerous condition, and this is called “cancer family syndrome.”6,7,12,14 These families show an increased incidence of adenocarcinoma, an earlier age of onset of malignancy, and an apparent vertical mode of transmission of the cancer. In view of these characteristics, the genetic background was assumed to be involved in carcinogenesis in these families and was accordingly analyzed. In order to analyze genetic factors, Lynch et al.8) examined HLA typing in “Family N,” which exhibits the cancer family syndrome. They presented data showing an association between HL-A2-HL-A12 haplotype and cancer in that family.

Moreover, immunologic abnormalities are suspected in cancer families in the light of various reports documenting subclinical immune defects in healthy relatives in cases of...
family accumulations of lymphoproliferative disorders,\textsuperscript{13} of gastric cancer,\textsuperscript{4} and of colon cancer.\textsuperscript{1}

We found a new example of familial large bowel cancer not associated with polyposis (family K). The purpose of this study was to analyze both immunological and genetic factors of affected and unaffected members of the family K.

**Materials and Methods**

**Patient Selection** A total of 17 individuals from this family accumulation of colon cancer was studied. Of these, two patients had a current or previous colon cancer and one had a rectal polyp, but all of them had received a curative operation prior to this study and showed no particular sign or symptom at the time of study. The remaining 14 individuals were unaffected. Clinically they were determined to be free of any malignancies or other significant diseases within 2 months prior to this study.

**Lymphocyte Blast Transformation Test**

Lymphocytes were cultured at a concentration of $10^6$ cells/ml in RPMI-1640 medium (Nissui Seiyaku Co., Ltd., Tokyo) that contained 100 units/ml of penicillin, 100 $\mu$g/ml of streptomycin, and heat-inactivated 20% fetal calf serum (v/v). Cultures were prepared in duplicate, and 2 $\mu$g/ml of PHA (phytohemagglutinin, Wellcome Reagents Ltd., Beckenham) was added. Cultures were incubated at 37°C in a 5% CO2 incubator for 72 hr. Transformation was assessed by applying a pulse of 1 $\mu$Ci of tritiated thymidine (The Radiochemical Centre, Amersham) per milliliter of culture for 3 hr before harvesting. Cultured cells were harvested by washing with cold saline on glass fiber filters. The acid-insoluble components were precipitated on filters with 10% trichloroacetic acid and counted using a Beckman liquid scintillation counter. Incorporation of thymidine was expressed as the mean cpm of duplicate cultures minus the mean cpm of the background.

**Normal Value of PHA Responsiveness**

A total of 46 unrelated normal individuals (28 males and 18 females) with ages ranging from 22 to 43 was tested for PHA responsiveness as described above. The family histories of each disclosed no evidence of a familial diathesis for malignancy. The mean cpm (incorporation of thymidine) for the PHA response of the peripheral lymphocytes from these individuals was 37,559, ranging from 13,036 to 94,300 cpm. A cpm more than 1 SD below this, namely, less than 18,560 cpm, was assumed to be indicative of decreased PHA responsiveness.

**Quantitation of T and B Lymphocytes**

Counting of T and B lymphocytes of peripheral blood was performed by a modification of Tachibana and Ishikawa’s method.\textsuperscript{15}

**Serum Immunoglobulin Levels**

The serum concentrations of IgM, IgG and IgA were measured with Partigen plates from Behringwerke Co., Ltd., Marburg.

**Serum Complement Assay**

Total complement activity was measured as hemolytic complement activity (CH50) according to Mayer’s method.\textsuperscript{9}

**Serum Carcinoembryonic Antigen (CEA) Levels**

The CEA levels in serum were measured by a radioimmunoassay using CEA-RIAKIT (DAINABOT Radioisotope Lab., Tokyo).

**HLA Typing**

HLA typing was done by the microdroplet lymphocyte cytotoxicity method.\textsuperscript{16}

**Statistical Analysis**

Statistical significance was calculated by means of the $\chi^2$ test. Yates' correction was used in the $\chi^2$ test. The relative risk (RR) was used for the associations between HLA and colon cancer occurrence.

**Results**

**History of The Family K**

A 16-year-old boy was admitted to our hospital in September 1977, with complaints of pains and tumor mass in the left lower abdomen. A proctoscopic examination and barium enema were performed, which revealed an annular lesion in the descending colon. The histological diagnosis for this tumor was a poorly differentiated adenocarcinoma. Unfortunately the patient died before the initiation of this study. Since the family history showed an accumulation of colon cancer, the relatives (II-1, 2, 3, 5, 8, III-1 and 3) of the proband were examined with barium enema and/or proctoscopy. Surprisingly, the father of the proband (II-1) proved to have an annular lesion in the descending colon. The histological diagnosis for this tumor was a poorly differentiated adenocarcinoma. Unfortunately the patient died before the initiation of this study. Since the family history showed an accumulation of colon cancer, the relatives (II-1, 2, 3, 5, 8, III-1 and 3) of the proband were examined with barium enema and/or proctoscopy. Surprisingly, the father of the proband (II-1) proved to have an annular lesion in the transverse colon without any clinical symptoms. Histological findings showed it to be a moderately differentiated adenocarcinoma. The characteristics of the family K are as follows. (1) Primary colon cancer without polyposis affects three generations in the family K. Fig. 1 shows that five out of 25 relatives had colon cancer and one had a
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Fig. 1. Pedigree of the family K.
I, II and III indicate generations of the family.

Table I. Site of Tumor and Histology of Colon Cancer Patients in the Family K

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age of onset</th>
<th>Sex</th>
<th>Site of tumor</th>
<th>Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-1</td>
<td>47</td>
<td>M</td>
<td>Descending colon</td>
<td>Adenocarcinoma</td>
</tr>
<tr>
<td>II-1</td>
<td>49</td>
<td>M</td>
<td>Transverse colon</td>
<td>Well diff. adenocarcinoma</td>
</tr>
<tr>
<td>II-4</td>
<td>32</td>
<td>M</td>
<td>Ascending colon</td>
<td>Mod. diff. adenocarcinoma</td>
</tr>
<tr>
<td>II-7</td>
<td>22</td>
<td>M</td>
<td>Descending colon</td>
<td>Poorly diff. adenocarcinoma</td>
</tr>
<tr>
<td>III-2</td>
<td>16</td>
<td>M</td>
<td>Descending colon</td>
<td>Poorly diff. adenocarcinoma</td>
</tr>
</tbody>
</table>

rectal polyp. Histological diagnoses of the affected members are shown in Table I.

(2) The age of onset of cancer in this family is about 20 to 30 years earlier than would be expected in control patients, as shown in Table II. Control patients consisted of confirmed colon cancer patients who live in the same area as this family. (3) All of the patients are male. (4) The proband was a 16-year-old boy, one of identical twins. The other twin shows no particular disorder so far. The following examinations were performed to check the immunological characteristics of the family members.

**PHA Responsiveness of the Family Members** Several immunological parameters have been defined for cell-mediated immune response in vitro. One of the representative parameters is the proliferative response of lymphocytes to PHA. A total of 17 relatives was tested for PHA response. Of the 17 relatives, two had colon cancer and one had a rectal polyp. However, all of them had already had a curative operation before this test and showed no signs or symptoms at the time of study. Table III shows that 1 of 2 affected members with colon cancer, one rectal polyp patient and 4 of 14 cancer-free relatives demonstrated significantly decreased PHA responsiveness \((P<0.0025)\). Carcinogenic risk seems to appear in the twenties in the family K. When limited to relatives over 20 years of age, five of seven relatives \((71\%)\) showed decreased PHA responsiveness \((P<0.001)\). On the other hand, among the relatives under the age of 20, only one of 10 relatives \((10\%)\) showed decreased PHA responsiveness.
Other Immunological Studies  Other immunological tests were carried out, including counting of T and B lymphocytes of peripheral blood, and determinations of serum immunoglobulin (IgM, IgG and IgA), serum complement activity (CH50) and serum CEA. These results are summarized in Table IV. All of them are within normal limits.

HLA Typing  Eighteen individuals from the family K were tested to determine their HLA types, and the HLA types of three relatives (I-1, II-4 and III-2), who died before this study, were deduced. As shown in Fig. 2, nine of 21 relatives had A9-Bw35 haplotype and 4 of them (44%) had a current or previous colon cancer. On the other hand, only one of 12 relatives (0.8%) who did not have the A9-Bw35 haplotype had colon cancer. We examined how many times more frequently colon cancer occurs in family members carrying the A9-Bw35 haplotype relative to family members lacking it, by calcu-
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Table IV. Other Immunological Parameters of the Family Members

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age</th>
<th>Sex</th>
<th>T/B cells</th>
<th>IgG (mg/ml)</th>
<th>IgA (mg/ml)</th>
<th>IgM (unit/ml)</th>
<th>CH50 (unit/ml)</th>
<th>CEA (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>II-1a)</td>
<td>49</td>
<td>M</td>
<td>85/15</td>
<td>1,300</td>
<td>563</td>
<td>66</td>
<td>35.6</td>
<td>1.4</td>
</tr>
<tr>
<td>II-2</td>
<td>47</td>
<td>F</td>
<td>60/38</td>
<td>1,560</td>
<td>363</td>
<td>204</td>
<td>40.0</td>
<td>1.2</td>
</tr>
<tr>
<td>II-3</td>
<td>45</td>
<td>M</td>
<td>42/62</td>
<td>1,300</td>
<td>231</td>
<td>120</td>
<td>35.6</td>
<td>1.9</td>
</tr>
<tr>
<td>II-5</td>
<td>40</td>
<td>M</td>
<td>76/26</td>
<td>1,560</td>
<td>202</td>
<td>86</td>
<td>40.0</td>
<td>1.0</td>
</tr>
<tr>
<td>II-7a</td>
<td>34</td>
<td>M</td>
<td>90/8</td>
<td>1,440</td>
<td>231</td>
<td>172</td>
<td>39.0</td>
<td>1.0</td>
</tr>
<tr>
<td>II-8</td>
<td>32</td>
<td>M</td>
<td>70/27</td>
<td>1,440</td>
<td>202</td>
<td>120</td>
<td>39.0</td>
<td>1.0</td>
</tr>
<tr>
<td>III-1</td>
<td>19</td>
<td>F</td>
<td>68/34</td>
<td>1,750</td>
<td>231</td>
<td>86</td>
<td>39.0</td>
<td>1.0</td>
</tr>
<tr>
<td>III-3</td>
<td>16</td>
<td>M</td>
<td>62/34</td>
<td>1,800</td>
<td>240</td>
<td>86</td>
<td>38.0</td>
<td>1.0</td>
</tr>
<tr>
<td>III-4</td>
<td>20</td>
<td>M</td>
<td>68/30</td>
<td>1,400</td>
<td>172</td>
<td>120</td>
<td>43.2</td>
<td>1.3</td>
</tr>
<tr>
<td>III-5</td>
<td>14</td>
<td>M</td>
<td>67/30</td>
<td>1,300</td>
<td>98</td>
<td>172</td>
<td>33.3</td>
<td>1.0</td>
</tr>
<tr>
<td>III-6</td>
<td>16</td>
<td>F</td>
<td>50/49</td>
<td>1,440</td>
<td>202</td>
<td>120</td>
<td>33.3</td>
<td>1.1</td>
</tr>
<tr>
<td>III-7</td>
<td>10</td>
<td>F</td>
<td>65/32</td>
<td>1,800</td>
<td>230</td>
<td>120</td>
<td>38.0</td>
<td>1.1</td>
</tr>
<tr>
<td>III-8</td>
<td>12</td>
<td>F</td>
<td>64/36</td>
<td>1,660</td>
<td>224</td>
<td>240</td>
<td>32.0</td>
<td>2.2</td>
</tr>
<tr>
<td>III-9</td>
<td>7</td>
<td>M</td>
<td>62/36</td>
<td>2,000</td>
<td>186</td>
<td>172</td>
<td>30.8</td>
<td>2.7</td>
</tr>
<tr>
<td>III-10</td>
<td>15</td>
<td>M</td>
<td>68/30</td>
<td>1,200</td>
<td>98</td>
<td>120</td>
<td>33.3</td>
<td>NT</td>
</tr>
<tr>
<td>III-11</td>
<td>6</td>
<td>M</td>
<td>60/42</td>
<td>1,000</td>
<td>98</td>
<td>114</td>
<td>33.3</td>
<td>NT</td>
</tr>
<tr>
<td>III-15</td>
<td>7</td>
<td>M</td>
<td>65/30</td>
<td>1,800</td>
<td>186</td>
<td>172</td>
<td>33.3</td>
<td>NT</td>
</tr>
<tr>
<td>III-16</td>
<td>4</td>
<td>M</td>
<td>65/32</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Control</td>
<td>3~55</td>
<td>M/F</td>
<td>66/34</td>
<td>1,329±288</td>
<td>235±76</td>
<td>142±64</td>
<td>35.6±5.18</td>
<td>0~5</td>
</tr>
</tbody>
</table>

a) Cancer patients  NT = not tested

Calculating the relative risk (RR). The relative risk is 8.8 in the family K. When limited to relatives over the age of 20, 5 relatives had A9-Bw35 haplotype and 4 of them (80%) had colon cancer. These results clearly demonstrate that the incidence of colon cancer is higher in relatives who had A9-Bw35 haplotype ($P<0.1$).

**DISCUSSION**

Various indicators have been defined for immune response in cancer patients. One of the representative indicators is the proliferative response of lymphocytes to PHA.11) There are several reports concerning the decreased response to PHA in cancer patients.3,8) On the other hand, families with multiple members affected by cancer of the large bowel or another organ have been reported,6,7,12,14) and it is of interest that a decrease of cell-mediated immunity was detected at a high rate among cancer-free relatives in those families.1,4,13) Accordingly, we carried out PHA reactivity tests on members of the present family K. As shown in Table III, one of two cancer patients who had received surgery showed reduced PHA responsiveness. It is of interest that 30% of the cancer-free relatives also showed decreased PHA responsiveness ($P<0.0025$). It should be noted that in a family with multiple cancer, the age at which cancer develops is lower than in the general population. Carcinogenic risk reportedly usually becomes greater in one's thirties.6,7,8,12) However, the risk seems to be greater from the twenties in the family K, as shown in Table II. Among relatives over 20 years of age, 5 of 7 relatives (71%) showed decreased PHA responsiveness ($P<0.001$). As for the cancer-free relatives over the age of 20, three of four relatives showed decreased PHA responsiveness ($P<0.001$). These results suggested a relationship between cancer occurrence in the family K and the decreased cell-mediated immunity.
On the other hand, HLA is of interest in connection with disease susceptibility. Immune response genes (Ir-genes) are assumed to be linked to HLA. Therefore, we considered that an HLA study would be a promising approach for immunogenetic investigation of the family K. Lynch et al. studied HLA genotype in “cancer family N,” in which colon cancer occurred predominantly. They reported that 20 of 21 colon cancer patients had HL-A2-HL-A12 haplotype. In contrast, the relatives who had A9-Bw35 haplotype tended to develop colon cancer in our family (RR=8.8). Four persons who did not have A9-Bw35 haplotype did not develop cancer. However, Lynch et al. suggested that the susceptibility gene may be linked to different HL-A haplotypes in other families.

As mentioned above, our family case has two characteristics. First, a high rate of decreased reactivity for PHA was observed even in cancer-free relatives, and second, some relationship is suggested between the A9-Bw35 haplotype and cancer patients. These findings suggest that immunogenetic elements may be implicated in the colon cancer occurrence in multiple members of the family K.

(Received Jan. 29, 1980/Accepted June 10, 1980)

References