41. Interferon-γ as a Marker for the Effective Cancer Immunotherapy with BCG-Cell Wall Skeleton

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Abstract: Immunotherapy with BCG-CWS (the cell wall skeleton from Bacille Calmette-Guerin) alone has been tried on the patients with various kinds of cancer, after surgical operation and/or chemotherapy. After the inoculation most of the patients revealed biological responses: skin reactions (erythema, induration and ulcer formation), physical signs, and cytokine induction in peripheral blood (G-CSF, IL-6 and IFN-γ). Among the patients, those who revealed distinct IFN-γ induction and/or strong skin reaction are alive in healthy state including a few cases of complete recovery. Those who showed no IFN-γ induction, even if they had weak skin reaction, died in a short period. Most of them were in terminal stage or had had repeated chemical or radiation therapy before immunotherapy.

These results suggest that nonspecific immunotherapy with BCG-CWS, if applied independently of any other therapy, will provide an extremely effective way to prevent relapse or occurrence of secondary cancer, and those who produce IFN-γ responding BCG-CWS inoculation can be regarded as perfect candidates for cancer immunotherapy under discussion.

Key words: Nonspecific immunotherapy; BCG-CWS; interferon-γ; antitumor effect.

Specific and nonspecific activation of the host immune system to reject cancer is an appealing idea. Although many researchers have challenged to this theme, it does not necessarily follow that Immunotherapy is accepted as one of the important methods for cancer treatment. In Japan, the nonspecific immunotherapy for cancer using BCG-CWS (the cell wall skeleton derived from Bacille Calmette-Guérin) was widely examined by Yamamura et al. in 1970's. In their randomized clinical trial, BCG-CWS was mostly used as supplementary to conventional therapies, and it failed to bring about good results. ¹)

The author assumed the following two questions about the unsatisfactory results of their clinical trial;
1. Was the clinical trial performed in proper way to give the real value of the immunotherapy?
2. Were the patients in the trial really qualified for the immunotherapy with BCG-CWS? If they were not, how could we decide well-qualified patients?

In order to answer these questions, the immunotherapy with BCG-CWS was tried on a new idea.

BCG-CWS² was kindly supplied by Dr. I. Azuma (Institute of Immunological Science, Hokkaido University). The preparation of oil-attached BCG-CWS was performed as previously reported,²) except for the ratio of mineral oil to BCG-CWS and the concentration of Tween 80 in saline: Drakeol 6VR, Pennsylvania Refining Co., Butler, U.S.A. was added, 2 drops per mg, to BCG-CWS powder, and the oil-attached powder was

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suspended in physiological saline containing 1.1% Tween 80. After sterilized by heating 30 minutes at 60°C, the suspension was inoculated intradermally on the external skin of upper arm according to the schedule which was composed of two phases; in the sensitization phase, 200 µg of BCG-CWS was inoculated weekly and, usually at 3 or 4 inoculations, patients' skin reactions (erythema, induration and ulcer formation) would reach to the maximum. Then the phase moved to the continuing phase, in which most of the patients were inoculated 100 µg of BCG-CWS monthly. Cytokines in serum were assayed by ELISA. IL-1 (α and β), IL-2, TNF-α, G-CSF, IFN-α and -γ were determined with assay kit from Otsuka Assay Co., and IL-6 and IL-8 were determined with the one from R & D Co.. Among these cytokines, only G-CSF, IL-6 and IFN-γ could be detected in peripheral blood after BCG-CWS inoculation.

In regard to the first question, there was a strong doubt about the program of the trial in that CWS was mostly used with various kinds of anticancer therapy which were supposed to impair our immune surveillance system. So the decision was made to use CWS on the patients independent of conventional therapies, just after initial operation or chemotherapy, if possible. Starting in 1974, an independent use of BCG-CWS was tried for the treatment of three patients, two with acute myelogenous leukemia (case 11 and 12 in

Fig. 1. Clinical course of a patient with colorectal cancer.

op : surgical operation.

: BCG-CWS inoculation and the numbers denote given dose in µg.

5-Fu : fluorouracil.

Ara-C : cytosine arabinoside.

MMC : mitomycin-c.
Table I. Cancer patients, their clinical and biological records and evaluation of immunotherapy with BCG-CWS alone

<table>
<thead>
<tr>
<th>Case (Age, sex)</th>
<th>Diagnosis (Histotype)</th>
<th>Initial Treatment (Maintenance)</th>
<th>Stage</th>
<th>IFN-γ in serum (pg/ml)</th>
<th>Skin* Reaction</th>
<th>Status (Duration)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (60, F)</td>
<td>Lung cancer (Large cell carcinoma)</td>
<td>Surgical operation (—)</td>
<td>II</td>
<td>ND†</td>
<td>2456.4</td>
<td>++</td>
</tr>
<tr>
<td>2 (68, F)</td>
<td>Pancreatic cancer (Adenocarcinoma)</td>
<td>Surgical operation (Chemotherapy)</td>
<td>IV</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>3 (42, F)</td>
<td>Uterine cervical cancer (Squamous cell carcinoma)</td>
<td>Surgical operation (Radiation)</td>
<td>IV</td>
<td>ND</td>
<td>ND</td>
<td>—</td>
</tr>
<tr>
<td>4 (45, F)</td>
<td>Lung cancer (Adenocarcinoma)</td>
<td>Surgical operation (Radiation)</td>
<td>IIIA</td>
<td>ND#</td>
<td>188.9 (ND)#</td>
<td>++</td>
</tr>
<tr>
<td>5 (33, F)</td>
<td>Colorectal cancer (Adenocarcinoma)</td>
<td>Surgical operation (—)</td>
<td>II</td>
<td>ND</td>
<td>146.8</td>
<td>++</td>
</tr>
<tr>
<td>6 (61, M)</td>
<td>Lung cancer (Adenocarcinoma)</td>
<td>Chemotherapy (Radiation)</td>
<td>IV</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>7 (74, M)</td>
<td>Gastric cancer (Adenocarcinoma)</td>
<td>Surgical operation (—)</td>
<td>IV</td>
<td>ND</td>
<td>240.9</td>
<td>++</td>
</tr>
<tr>
<td>8 (51, F)</td>
<td>Ovarian cancer (Adenocarcinoma)</td>
<td>Surgical operation (Chemotherapy)</td>
<td>Ic</td>
<td>ND</td>
<td>80.5</td>
<td>++</td>
</tr>
<tr>
<td>9 (70, M)</td>
<td>Lung cancer (Large cell carcinoma)</td>
<td>Surgical operation (Chemotherapy)</td>
<td>IV</td>
<td>ND</td>
<td>ND</td>
<td>—</td>
</tr>
<tr>
<td>10 (66, M)</td>
<td>Colorectal cancer (Adenocarcinoma)</td>
<td>Surgical operation (Chemotherapy)</td>
<td>IV</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>11 (35, F)</td>
<td>Acute leukemia (Promyelocytic)</td>
<td>Chemotherapy (Chemotherapy)</td>
<td>§</td>
<td>ND¶</td>
<td>297.2¶</td>
<td>++</td>
</tr>
<tr>
<td>12 (30, M)</td>
<td>Acute leukemia (Myeloblastic)</td>
<td>Chemotherapy (—)</td>
<td>§</td>
<td>ND¶</td>
<td>107.6¶</td>
<td>++</td>
</tr>
</tbody>
</table>

*: —, no skin reaction, +, ulcer only, ++, erythema (dia. more than 20 mm), induration and ulcer.
†: ND, not detected (less than 15.0 pg/ml).
#: (ND), 6 month later after the x-ray irradiation.
§: There is no expression corresponding to stage grouping.
¶: When the author treated case 11 and 12, determination of IFN-γ was impossible.
These data were examined recently.

Table I) and one with colorectal cancer, after obtaining their consent.

Fig. 1 shows an early part of the clinical course of the patient with relapsed colorectal cancer accompanied by peritonitis carcinomatosa. The patient first complained of severe pain in abdomen like ileus in September 1975, at the age of 72. After being diagnosed as colorectal cancer, she had a surgical operation at Toyohashi City Hospital, Toyohashi,
Japan. After the operation, as shown in Fig. 1, she was treated with conventional combined chemotherapy (fluorouracil, cytosine arabinoside, and mitomycin-c) and her clinical course was followed by serum carcinoembryonic antigen (CEA) level. Though she was doing well with CEA level decreasing after the operation, her clinical course was reversed 2 months later with CEA level beginning to increase. Further she complained of full feeling in abdomen and anorexia, and the sign of ascites appeared. Laboratory examination disclosed hypoproteinemia and the presence of peritonitis carcinomatosa. So the chemotherapy was discontinued and immunotherapy with BCG-CWS alone was initiated. After 5 months her ascites disappeared and serum CEA level decreased to the normal. After that she completely recovered and her CEA level was maintained within normal range until her death. Immunotherapy continued about 8 years and she completed the natural span of life at the age of 87. Other two patients, one of them had already had a relapse at the start of immunotherapy, completely recovered by the immunotherapy with BCG-CWS alone and are still alive having no secondary cancer. These data are the examples to show that some cancer patients could be cured, even if they had a relapse, by BCG-CWS alone.

In order to examine the second question, recently analyses were made on various kinds of biological responses induced by the immunotherapy with BCG-CWS. Twelve patients with 4 lung, 2 colorectal, 1 pancreatic, 1 uterine cervical, 1 gastric and 1 ovarian cancer and 2 myelogenous leukemia, of whom more than half (7/12) were at stage III to IV, were treated with intradermal inoculation of BCG-CWS alone, 100 or 200 µg monthly (Table I). The informed consent in writing was obtained from these patients. After the inoculation, most of the patients revealed some biological responses: skin reaction, low grade fever, alteration of laboratory indices (white cell counts and its differentials, and C-reactive protein), and cytokine induction in peripheral blood (G-CSF, IL-6 and IFN-γ). Among these biological responses, IFN-γ induction and skin reaction are mainly related with antitumor activity: as shown in Table I, of these 12 cases, 7 patients showed a distinct IFN-γ induction (more than 15.0 pg/ml) and/or strong skin reaction, and most of them (6/7) are alive in healthy state including two cases of complete recovery. Those who showed no IFN-γ induction (5/12), even if they had weak skin reaction, died in a short period. Most of them were in terminal stage or had had repeated chemical or radiation therapy before immunotherapy. So, it is recommended that, if the patients do not reveal any expected biological responses including IFN-γ at the first inoculation in continuing phase, immunotherapy may be replaced by original or other conventional therapies. Case 4 showed a marked induction of IFN-γ initially, but ceased to show it 6 months later after the x-ray irradiation for neck bone metastasis, and died after 1 year.

As for the precise mechanism of the immunotherapy with BCG-CWS, there are many unsolved problems. In the present study, it was found that BCG-CWS induced IFN-γ, especially in patients who showed good prognosis. In contrast, all patients who could not induce it were dead. This fact might have some relation with the data that T-lymphocyte from 13 of 14 AIDS patients completely failed to secrete IFN-γ upon stimulation with specific microbial antigen. So IFN-γ seemed to be one of the most important cytokines in immune regulation essential for the antitumor effect. Though Yamamura’s group had tried cancer immunotherapy using BCG-CWS or Nocardia-CWS, this therapy finally failed to get the approval of the Ministry of Health and Welfare of the Japanese Government. The most important reason of this failure may have been in the inadequate selection of the subjects; in their trial, the study patient group perhaps included many unresponsive subjects to CWS who were having combined chemotherapy, radiation therapy or were in far advanced stage of cancer. Most of these patients might be in the worst immunological condition for cancer immunotherapy. In order to evaluate the value of...
cancer immunotherapy correctly, immunotherapy, as suggested in the present study, should be performed independently of other conventional therapies and on the patients who produce IFN-γ responding BCG-CWS inoculation. Since chemotherapy has still been regarded as the most powerful tool for cancer treatment in Japan, or perhaps in other countries, too, most patients are given anticancer drug just after initial operation. As the independent nonspecific immunotherapy under discussion grows in reliability with increased number of cases, it will be approved, in near future, as the most important and natural maintenance therapy for cancer patients.

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References