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Abstract

With the recent advances in the immunological surveillance system, an understanding of the role of host immunity has become essential to the management of carcinogenesis, tumor proliferation, recurrence and metastasis. Although it is important to continue chemical and surgical treatment of cancer, support of the anti-tumor immune system of the host should also be considered. Long term remission has been reported in leukemia by treating with BCG after chemotherapy whereas surgical treatment is usually more effective in preventing cancer recurrence in digestive organ cancer. The first step is extirpating the tumor as thoroughly as possible and the second step is chemo-immunotherapy. Cancer immunity, however weak, constitutes the basis for other treatments in selectively attacking cancer cells remaining after surgery, chemotherapy or irradiation. Immunotherapy should thus not replace chemotherapy or radiotherapy, but these methods should be employed in combination to attain more favorable results.

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CANCER IMMUNOTHERAPY WITH SURGERY

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Abstract. With the recent advances in the immunological surveillance system, an understanding of the role of host immunity has become essential to the management of carcinogenesis, tumor proliferation, recurrence and metastasis. Although it is important to continue chemical and surgical treatment of cancer, support of the anti-tumor immune system of the host should also be considered. Long term remission has been reported in leukemia by treating with BCG after chemotherapy whereas surgical treatment is usually more effective in preventing cancer recurrence in digestive organ cancer. The first step is extirpating the tumor as thoroughly as possible and the second step is chemo-immunotherapy. Cancer immunity, however weak, constitutes the basis for other treatments in selectively attacking cancer cells remaining after surgery, chemotherapy or irradiation. Immunotherapy should thus not replace chemotherapy or radiotherapy, but these methods should be employed in combination to attain more favorable results.

Tumor proliferation should be considered in terms of its inter-relationship to host resistance. So far, the role of host resistance has been vague with treatment being directed only at the tumor so that postoperative recurrences seemed to be entirely due to inability of the operating surgeon to extirpate all tumor tissue. We have, however, encountered cases of progressive cancer where metastasis appeared to have been triggered by the operation, and seemed to hasten death, whereas other cases of partial tumor extirpation showed long remission or survival. Moreover, occasional cases of spontaneous remission are reported. These varied responses indicate that host resistance is involved in tumor proliferation.

With the recent remarkable advances in immunology, it has become clear that host resistance consists of an immunological surveillance system which is centered on T-lymphocytes. Thus immunological support of the host appeared as a rational mode of cancer treatment. One problem is assessing to what extent the host can reject cancer. Numerous other factors include the immune capacity of the host which resides in the Ia gene and tumor antigenecity. Klein *et al.* (1) showed that up to 10^6 viable autochthonous methylcholanthrene-induced tumor

cells were rejected, but at above 10^7 cells, tumor death resulted in mice regardless of the degree of immunization by X-irradiated autochthonous tumor cells. Mathé *et al.* (2) reported that, in their experiments with F_1 mice and transplantation of L-1210 leukemic cells resistant to all treatment, the number of tumor cells rejected in untreated F_1 mice was low, but that up to 10^5 cells were rejected when the mice had been previously well immunized with either BCG or irradiated L-1210 cells. The results indicate enhanced antitumor activity as a consequence of immunotherapy.

In human terms, this is equivalent to 6×10^6 cells or 0.6g of tumor mass. Mathé *et al.* (3) after inducing remission in 30 childhood cases of acute lymphocytic leukemia by intensive chemotherapy divided them into four groups: 10 untreated controls (Group I), 8 cases given BCG (Group II), 5 cases given subcutaneous injection of leukemic cells (Group III) and 7 cases given injection of leukemic cells mixed with BCG (Group IV). These investigators administered 150mg of viable Pasteur BCG by the scratch method every 4 days for the first month, and thereafter once weekly. In Group I and Group III, recurrence occurred in all cases within 130 days, but in Groups II and IV a long period of remission ranging from 4.5 to 7 years was attained.

Immunotherapy conducted after the tumor decreased in size was definitely a more successful method of treatment. Chemotherapy is required for progressive solid tumors where remnants are apt to occur and for leukemia of the fluid form. One disadvantage of present chemotherapy methods, however, is the lack of selective action, and, at present, there is a limit to the percentage of cancer cells killed by chemotherapy which is unrelated to the original number of cancer cells (4). That is, the dose of anticancer agent necessary to decrease 10^{12} tumor cells to 10^9 cells (1kg→1g) should theoretically be the same as that required to decrease 10^9 cells to 10^6 cells (1g→1mg). Estimates have shown that even when an anticancer agent is administered at the maximum tolerant dose, about 10^9 cancer cells survive. Moreover, due to lack of selectivity, the bone marrow and lympho-reticuloendothelial systems are disturbed and this results in depression of the immune system, so that subsequent immunotherapy is less successful. With solid tumors, surgical intervention may cause a slight disturbance in the immune system but much less so than an anticancer agent. The tumor mass is thus reduced (reduction surgery), and with subsequent immunochemotherapy, a higher success rate than that reported for leukemia by Mathé may be anticipated. We will discuss our findings on both how cancer patient immunity is affected by surgery and the role of postoperative immunotherapy.

Indices of Immune Capacity in Cancer Patients

The ability to produce humoral antibody is generally maintained to the terminal stage in patients with solid tumors. The problem is the cell-mediated

immunity. In examining cell-mediated immunity of cancer patients, two factors require examination: (a) specific cell-mediated immunity that, recognizing tumor specific transplantation antigen (TSTA) and tumor specific antigen (TSA), reacts against them, and (b) non-specific cell-mediated immunity that represents the general biological activity and the numbers of lymphocytes. Such examinations should not aggravate the condition of the patient and should be reproducible. Skin tests with recall antigens such as pure protein derivative (PPD) and dinitrochlorobenzene (DNCB) are frequently used *in vivo* as an index of non-specific cell-mediated immunity. *In vitro* tests generally used include: blastformation of T-lymphocytes against phytohemagglutinin (PHA) and concanavalin A, and the ratio of peripheral T-cells and B-cells. The parameters of specific cell-mediated immunity are mainly *in vitro* tests. *In vitro* tests include: lymphocytotoxicity tests where lymphocytes are added to autochthonous cancer cells or to established homologous cancer cells with cross-antigens; colony inhibition tests CI-test); and estimation of lymphokines liberated from immunized lymphocytes in response to tumor antigens, especially assay of macrophage migration inhibition factor (MIF) and leukocyte migration inhibition factor (LIF), and estimation of the blastformation rate of peripheral blood lymphocytes to autochthonous tumor antigens (mixed lymphocyte-tumor reaction, MLTR). When these tests are positive, the presence of specific cell-mediated immunity, TSTA and/or TSA is indicated.

If target cancer cells and their antigens are treated with the cancer patient serum prior to the addition of lymphocytes, blocking factors may be measured in serum. It is thus possible to demonstrate specific cell-mediated immunity and the presence of cross-antigens in human cancer of the same histological type (such histological types include melanoma, leukemia, neuroblastoma, colon cancer, and gastric cancer).

Preoperative Cell-mediated Immunity in Cancer Patients

In both animal and human ccancer (5–7) specific cell-mediated immunity develops first in the regional lymph nodes and such immunity grows stronger as the tumor progresses, and as the tumor increases beyond a certain level, this specific immunity weakens in the regional lymph nodes and appears in more distant lymphatic tissues and peripheral blood lymphocytes (5–7). Specific cell-mediated immunity in even peripheral blood lymphocytes disappears by the terminal stage of cancer. In lymph node cells of progressive gastric cancer, the cytotoxicity is stronger at the distal mesentery lymph nodes than at the regional lymph nodes. O'Toole *et al.* (8) have classified urinary bladder cancer into stage T_1 to T_4: in mucosa (T_1), infiltration stopping at the muscle layer surface (T_2), infiltration into deep muscle layer but the tumor is mobile (T_3) and infiltration out of the bladder wall and immobile (T_4). They reported that positive lympho-

cytotoxicity was found in 88% of T_1-T_2 cases, while it was 41% in T_3-T_4 cases. Positive MIF in peripheral blood lymphocytes was found in gastric cancer patients in 6 of 14 Stage I cases (42.9%), in 13 of 23 Stage II cases (56.5%), in 28 of 55 Stage III cases (50.9%) and in 14 of 44 terminal Stage IV cases (31.5%). Even with colon cancer, the tendency was similar. McCoy *et al.* (9) found that LIF was positive in 11 of 15 Stage I cases (75%) of melanoma with localized tumor, in 16 of 27 Stage II cases (50%) with regional lymph-node metastases and in 21 of 37 Stage III cases (57%) with distal metastases.

Non-specific cell-mediated immunity was inversely proportional to the advance of cancer. The positive rate of both the tuberculin test and DNCB reaction decreased along with the progress of gastric cancer and colon cancer. Sheep red blood cell rosette-forming cells (T-cells) also tended to decrease in progressive gastric cancer. A clear-cut mutual relationship was especially present between PHA-blastformation rate and cancer progress, and a successful operation for cancer appeared possible if the preoperative blastformation rate was over 40% in gastric cancer and colon cancer. If the blastformation rate was 30-40%, a non-curative operation was indicated, and if the rate was under 30% and DNCB test negative, a palliative operation was likely. Our own data indicate that a PHA-blastformation rate of 40% is the critical point.

Reasons for Decreased Cell-Mediated Immunity in Progressive Cancer

Until the tumor reaches a certain size, lymphocytes have positive lymphocytotoxicity. The mechanisms of cancer cell rejection is complex. Cancer cells are attacked by many different cells, such as effector T-cells that adhere directly to cancer cells and destroy them, B-cells and macrophages with Fc-receptors on their cell surface, and small lymphocyte-like K cells on whose cell surface there is no demonstrable immunoglobulin. The last three types of cells are thought to have humoral antibody controlled by the T-lymphocyte or with lymphokines produced by T-lymphocytes, and attack cancer, and the T-lymphocytes probably play the main role in the rejection of cancer cells (10). As cancer advances, an excessive amount of tumor antigens is liberated surpassing the disposal capacity of the host. Such antigen conjugates with the receptor of the T-lymphocyte and neutralizes it, and by further combining with the antibody produced by B-cells, forms an antigen-antibody complex (a blocking factor). The blocking factor combines with the immune T-lymphocytes and cancer cells, and thus inhibits the cytotoxic activity of the immune T-lymphocytes. There is also an increase of non-specific immunosuppressive factors, such as α_2-globulin, carcino-embryonic antigen and toxohormone produced by the tumor itself in progressive cancer. The decrease of cell-mediated immunity in progressive cancer may arise from the large tumor itself, as well as from the spleen with numerous B-cells that produce the antibody and with suppressor cells (Fig. 1).

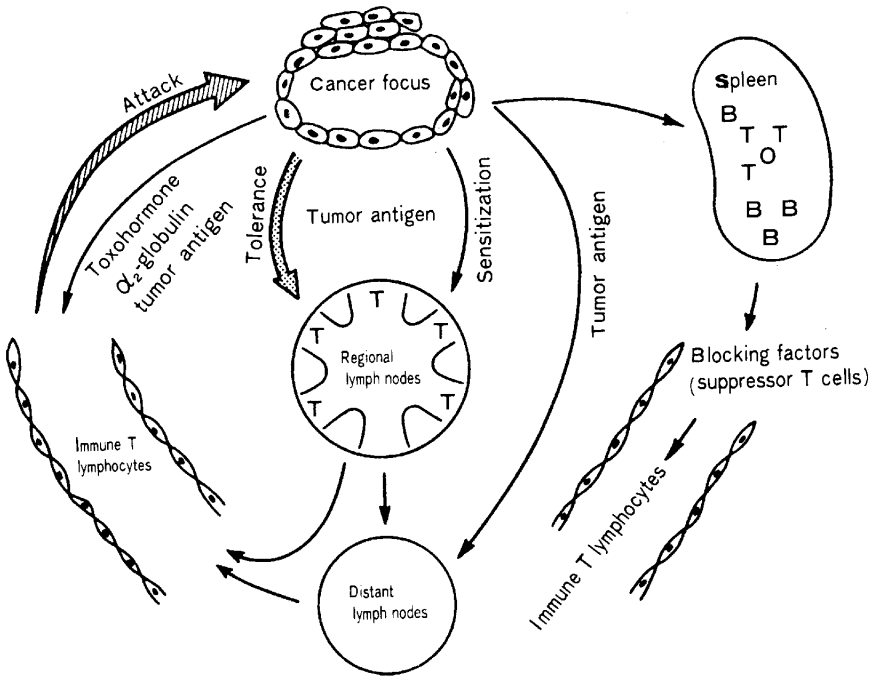

Fig. 1. The progress of cancer and changes in cell-mediated immunity. In a relatively early stage of cancer, tumor antigen reaches the regional lymph nodes in adequate doses and generates immune T-cells that attack the cancer focus. With the growth of tumor, an overdose of tumor antigen makes the immune T-cells tolerant and blocking factor, a complex of antibody and tumor antigen, specifically inhibits the activity of immune T-cells. Massive toxic substances, such as toxohormone are produced by the cancer focus that inhibit the T-cells.

Postoperative Cell-Mediated Immunity in Cancer Patients

Postoperative cell-mediated immunity may be suppressed by anesthesia, surgical intervention and tumor extirpation. It is already known that phagocytosis of the reticuloendothelial system is markedly decreased after hemicolectomy in dogs (11). Slade *et al.* (12) have observed changes of immunity *in vitro* and *in vivo* before, during and after nephrectomy in 12 renal donors. The average time for anesthesia was 4 hr and the average duration of operation was 3 hr. Anesthesia was induced with pentothal in all cases, and for maintenance, fentanyl was given to 10 cases, and halothane or ethrane was administered to another two cases. As *in vitro* criteria, peripheral blood lymphocyte counts, T-and B-cell counts, PHA-blastformation rate and one-way MLC (mixed lymphocyte culture) tests were used. As soon as anesthesia took effect, immunity began to weaken and reached a minimum level at 24–48 hr after the operation, and by the fifth postoperative day the immunity returned to approximately the preopera-

tive level. For *in vivo* criteria Slade *et al.* used skin tests with recall antigens. The skin reactions started to decrease gradually at induction of anesthesia and tended to reach a minimal level by the fifth post-oparative day. It required 10–14 days for complete recovery of the skin reaction by SK-SD and it took over 2–3 weeks with mumps and candida. There was no change in serum globulin level. It is worth noting that a time difference was present between immunity changes *in vivo* and *in vitro*.

Eilber and Morton (13) extirpated tumors as thoroughly as possible in sarcoma and melanoma cases and started immunotherapy with BCG at 3–6 postoperative weeks while continuing the DNCB test every two weeks. They immunized sarcoma cases with a mixture of BCG and established sarcoma cells (10^8 cells). The prognosis was good in melanoma cases with positive DNCB tests preoperatively. Recurrence was seen in only 3 of 21 cases (14%) that turned positive after the operation out of 51 cases negative before the operation, recurrence was seen in 22 of 30 cases (74%) that were negative throughout, and recurrence was present in all seven cases that turned negative after the operation. Similar results were obtained even with sarcoma cases. Hersh *et al.* (14) administered chemotherapy to adults with acute leukemia where they had repeated skin tests with dermatophytin O, candida and mumps. The skin reactions decreased once after chemotherapy but recovered within 6 months and were maintained positively in cases having remission periods over one year. However, such reactions decreased one month prior to recurrence in cases having recurrence within one year.

In following up the PHA-blastformation rate of peripheral blood lymphocytes after the operation, most cases showed a low value up to the second postoperative week, but the rate became stable thereafter. Comparisons of the blastformation rate before and 4 weeks after the operation indicated that the postoperative rate tended to rise slightly in 12 cases of benign diseases and in 42 cases receiving curative cancer operation, while the rate was decreased further from 34.2% to 28.1% in cases undergoing palliative operations. Ten of 15 cases of gastric cancer with preoperative blastformation rates of less than 40% showed rates above 40% at 4 postoperative weeks after the curative operation, and the rates were over 40% in all cases with favorable clinical courses over a period of one to two years. With recurrent cases, the blastformation rate on the way to recovery fell acutely 2–3 months before clinical confirmation of recurrence (Fig. 2). In 70 cases with favorable postoperative clinical courses and 99 cases with recurrences after discharge, the blastformation rate was 51% and 29% respectively. As long as the tumor was completely extirpated, the T-lymphocyte function recovered gradually even in advanced cancer.

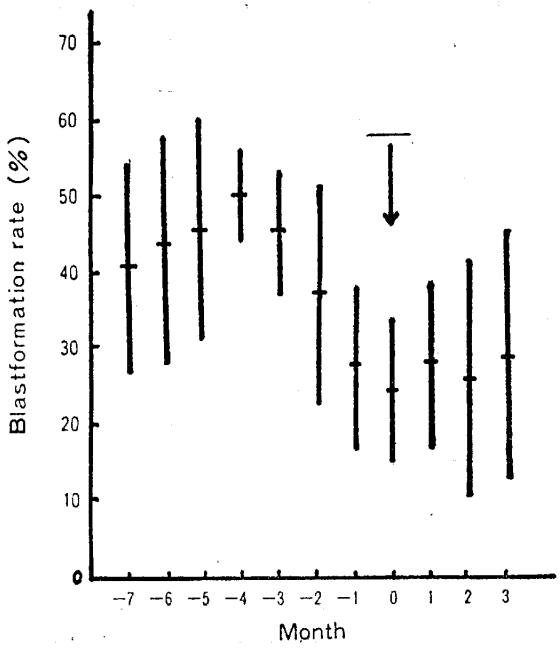

Fig. 2. Changes in PHA-blastformation rate before and after the postoperative recurrence of gastric cancer. The arrow indicates the time of the recurrence. PHA-Blastformation rate decreased 2 months before clinical confirmation of recurrence.

Tumor extirpation and blocking factors. Hellström *et al.* (15) found in isotransplantation of methylcholanthrene-induced (MC) sarcoma of mice that the blocking factor decreased at four days after tumor removal. Heppner (16) transplanted spontaneous mammary cancer maintained by serial passage in syngeneic mice and divided these mice into three groups: complete tumor extirpation 3–10 weeks after transplantation when the tumor became palpable, sham-operated and untreated. These investigators estimated the tumor-colony formation-inhibitory activity (CI activity) of lymph node cells and the blocking factor in their sera at 10–15 postoperative days when the tumors of the latter two groups grew to 10–20 mm in diameter. CI activity was positive in 12 of 13 cases of the extirpated group, in 12/15 cases of the sham-operated group and in 7/17 cases of the untreated group, giving the impression of a rise in cell-mediated immunity in the sham-operated group. The blocking factor was positive: in 5/6, 2/4 and 2/5 cases, respectively, in postoperative days 1–5; in 4/13, 5/9 and 6/10 cases, respectively, in postoperative days 10–15; and the blocking factor decreased significantly along with the lapse of time as 5/6 to 4/13 cases in the extirpated group. Positive lymphocytotoxicity was present in both the isotransplanted polyoma

tumor group and the tumor-extirpated group, while the antitumor activity was so strong as to reject the replanted tumor in the tumor-extirpated animals not demonstrating blocking factor, compared with tumor-bearing animals with blocking factor (17). A deblocking factor that neutralized the blocking factor was produced in serum within one week after polyoma tumor extirpation. There is no report of a blocking factor with a lapse of time in human cancer, but blocking factor was found in extensive, progressive cancer patients. The blocking factor was obliterated in serum of patients undergoing curative operations (18). In extensive melanoma-metastasis cases positive blocking factor was found in 87% of cases, while it was negative in all cases of localized melanoma and during remission (19). Moreover, it is said that complement dependent antibody with antitumor activity appears in completely extirpated localized melanoma (20). Maluish and Halliday (21) stated that if melanoma is sufficiently removed, blocking factor is demonstrated in the second postoperative week but disappears in the seventh week.

Tumor extirpation and lymphocytotoxicity. In 1966 Mikulska *et al.* (22) found no inhibition of tumor growth in spleen cells of mice bearing benzopyrene-induced sarcoma, but anti-tumor activity appeared in spleen cells three weeks after tumor extirpation, with an *in vivo* neutralization test of implanting a mixture of spleen cells and sarcoma cells to other normal syngeneic mice. Barski and Youn (23) found positive CI-activity in peritoneal cells (40% lymphocytes and 57% macrophages) of mouse isotransplanted subcutaneously with Rauscher virus-induced sarcoma on post-transplantation day 9 when the tumor was 2–5 mm in diameter, but CI-activity turned negative on day 40 when the tumor reached 20 mm in diameter. It became positive 12 days after extirpation on transplantation day 17 when the tumor was 10–15 mm in diameter, and it was still positive 56 days later. Hellström *et al.* (24) reported that peripheral blood lymphocytes maintained antitumor activity for several years and to a maximum of 29 years after remission of melanoma and colon cancer. On the other hand, O'Toole *et al.* (25) found that the cytotoxicity of peripheral blood lymphocytes disappeared by 4–8 postoperative weeks in cases where the urinary bladder cancer was completely extirpated, but cases with residual tumor tissues showed strong cytotoxic activity even at the fifth postoperative week. Irradiation at 4,000R at 4–6 weeks before surgery reinforced cell-mediated immunity, and the cytotoxic activity was maintained for 3–6 postoperative months. This is probably due to liberation of antigen from the tumor which became necrotic by irradiation.

The foregoing are reports concerned with cases where the blocking factor was mainly decreased by tumor extirpation and where the lymphocytotoxicity rose. *In vivo* the antitumor activity was reinforced. It is not unusual, however, to observe metastasis and aggravation of tumor following operation because cell-

mediated immunity is decreased merely by anesthesia or surgical intervention alone. According to Greene and Harvey (26), lymphoma cells were demonstrated in circulating blood of most hamsters transplanted s.c. with these tumor cells 7 days earlier, but these hamsters survived for one month without metastasis. However, when the tumor was extirpated on the 13th day after transplantation, metastasis occurred in 62.7% of hamsters. Stjernswärd (27) also observed the decline in concomitant immunity and the ready growth of the retransplanted tumor after radical operation in mice bearing MC-induced sarcoma. Gershon and Carter (28, 29) also found in homotransplantation of lymphoma to hamsters that when the tumor was extirpated one week after transplantation, metastasis formation was accelerated, but when tumor was replanted, the immunity recovered. The fact that the presence of the main tumor inhibited metastasis was also shown with MC-sarcoma in mouse (30).

Tumor extirpation, macrophage migration inhibition factor (MIF) and leukocyte migration inhibition factor (LIF).

Many reports indicate that MIF and LIF may be used as criteria for specific cell-mediated immunity and that they decrease after tumor extirpation. MIF and LIF differ in molecular weight and must have different biological activity but clinically they might be taken as the same thing without much inconvenience. McCoy *et al.* (9) stated that LIF in melanoma decreased after operation but returned practically to the normal level within 11–30 days, and even one year later was positive in 16 of 27 cases (59%). Cochran *et al.* (31) similarly reported that LIF declined within 4–5 days after the operation in practically all cases of melanoma and mammary cancer but recovered to the preoperative level 6–22 days later. Andersen *et al.* (32) obtained contrary results in that LIF disappeared one month after the operation in mammary cancer cases with a favorable course without recurrence. Lurie *et al.* (33) observed that LIF turned negative in all seven cases with localized colon cancers which were completely extirpated at the second postoperative month. In our gastric cancer cases, the positive MIF response of peripheral blood lymphocytes before the operation disappeared at one month after the operation in 7 of 9 cases of Stages I and II where the main lesion including the metastatic foci were believed to have been removed radically. On the other hand, MIF which was negative prior to the operation became highly positive after the operation in 13 of 17 cases of Stage IV where the tumor had been thoroughly extirpated (Table 1).

To clarify the relationship between the tumor mass and MIF, we implanted s.c. MC-sarcoma to syngeneic mice, extirpated tumor weekly as radically as possible and periodically assayed the MIF changes in the regional axillary lymph nodes. Where the tumor corresponded to Stage I and II of gastric cancer, the MIF decreased rapidly in the group undergoing tumor extirpation one week after

TABLE 1. CHANGES IN MIF VALUES BEFORE AND AFTER OPERATION IN GASTRIC CANCER PATIENTS

Stage	Before operation	One month after operation	Patient ratio	%
I and II	+	−	7/9	77.8
	−	+	4/12	33.3
III	+	−	7/11	63.6
	−	+	7/10	70.0
IV	+	−	1/6	16.7
	−	+	13/17	76.5

In Stage I and II 77.8% of patients with positive MIF before the operation turned negative after the operation, and 33.3% of patients with negative MIF turned positive after the operation. In Stage IV patients with tumor extirpated thoroughly, 16.7% of patients turned from positive to negative MIF and 76.5% of patients turned from negative to positive MIF. The MIF response between Stage I and II versus Stage IV was statistically significant.

tumor transplantation. When the tumor was equivalent to Stage IV, the MIF activity quickly became positive with tumor extirpation three weeks after transplantation, but disappeared rapidly on recurrence (Fig. 3). Recurrence occurred in 14% of mice in the former group and in 90% of the latter group.

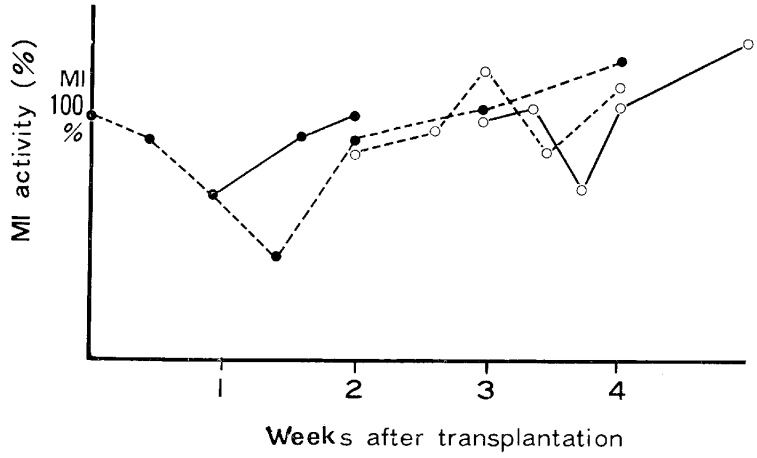

Fig. 3. Tumor extirpation after transplantation of MC tumor and MIF of regional lymph nodes. ●---●, Control; ●——●, extirpation one week later; ○---○, extirpation two weeks later; ○——○, extirpation three weeks later; MI, macrophage migration inhibition. In the control group the MIF activity reached maximum at 10 days and decreased as the tumor proliferated. However, at tumor extirpation one week after transplantation, MIF turnd negative rapidly. At tumor extirpation 3 weeks later, MIF turned positive rapidly, and thereafter it rapidly became negative with tumor recurrence.

When mitomycin C treated Ehrlich cancer cells were transplanted to six groups of mice in six inoculation sizes from 10^3 to 10^8 tumor cells, and the MIF of the regional axillary lymph nodes estimated periodically after transplantation, the greatest MIF activity was observed in the group receiving 10^5 tumor cells (Fig. 4).

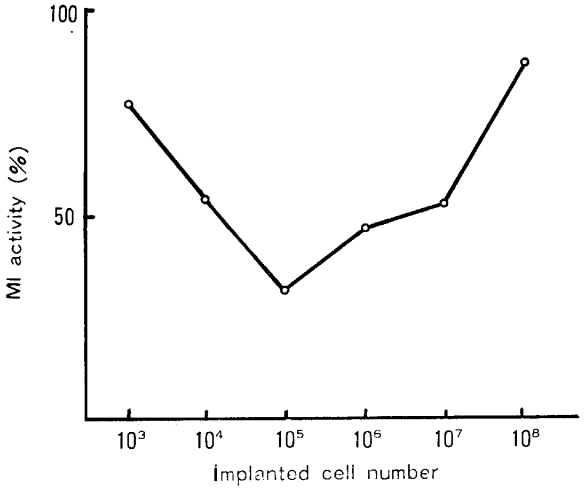

Fig. 4. Number of mitomycin-treated Ehrlich cancer cells subcutaneously implanted and the MIF at the regional lymph nodes at day 10 after implantation. The figure shows the relationship between the tumor mass and MIF. The greatest MIF activity was observed in the group receiving 10^5 Ehrlich cancer cells.

Akiyama (34) also stated that cases including gastric cancer whose MIF turned negative in 1–1.5 postoperative month were at relatively early cancer stages. These findings indicate that a certain quantity of tumor tissue or tumor antigen is necessary for positive MIF activity. Such findings lead us to a working hypothesis that postoperative immunotherapy using the resected tumor is necessary in patients undergoing complete tumor removal.

Postoperative Immunotherapy

Summarizing the immunity changes in cancer patients before and after the operation, it may be said that non-specific cell-mediated immunity gradually recovers as long as the tumor is completely extirpated, but there is a danger of rapid obliteration of specific cell-mediated immunity. Postoperative specific cell-mediated immunity should play a role in the rejection of remnant cancer selectively and in prevention of recurrence. However, such rejection power is not as strong as earlier mentioned. Consequently, it is necessary at an early stage of cancer to provide immunotherapy using resected autochthonous tumor tissue, and

in progressive cancer to diminish the residual tumor tissues. Anticancer agents should be administered carefully preserving the immune system with some immuno-potentiators to increase cell-mediated immunity. There are nonspecific and specific methods in immunotherapy, and the most effective method available today is immunotherapy centering around BCG. In the NIH protocol of immunotherapy, the viable BCG, bacterial component, anaerobic corynebacterium and levamisole are used. Cancer types and immunization methods are indeed diverse, but immunotherapy against gastric cancer is rare. Recently, Falk *et al.* (35) sprayed 2 mg BCG in the abdominal cavity before closure of the abdominal wall, and 120 mg BCG was given orally every week combined with 5-FU and endoxan for 8 weeks to 37 gastric cancer or colo-rectal cancer cases that received simple laparotomy. They found that such treatment was especially effective in colon cancer in which the average survival of 19 cases was 13.2 months, and within this group, survival of liver metastasis patients was 12.8 months, being about 2.5 times greater compared with 146 days of the control group without immunotherapy.

We have conducted immunotherapy in our patients with BCG or BCG-CWS made by Yamamura and Azuma of Osaka University. After i. d. isotransplantation of MH 134 hepatoma cells to C3H mice, the animals were divided into five groups: (a) the untreated control group, (b) the group with tumor extirpation two weeks after transplantation, (c) the group with tumor extirpation and i.d. injection of 4 mg BCG, (d) the group with tumor extirpation and i.d. injection of mitomycin treated MH 134 cells and (e) the group with tumor extirpation and i.d. injection of a mixture of mitomycin treated MH 134 cells plus BCG. In the control group (a) MIF in the regional lymph nodes reached a maximum on the sixth day of transplantation and decreased as the tumor grew larger and disappeared within two weeks. In groups (b) and (d) MIF underwent about the same changes as in group (a); in group (c) MIF showed slight reinforcement; and in group (e) receiving combination treatment, MIF continued to grow stronger for about 20 days. The 50% survival days of each group was: 24, 49, 47, 47 and 93 days, respectively. Group (e) immunized with BCG plus tumor cells simultaneously with tumor extirpation showed a significant prolongation in survival time and sustained reinforcement of MIF (36).

In the period between November 1973 to November 1975, immunotherapy was conducted on our gastric and colon cancer patients where at least the main tumors could be extirpated by the following protocol. Free tumor cells were isolated from 0.5 g of homogenized autochthonous tumor tissue and were treated with 12.5 mg/ml of mitomycin C for 30 minutes. Such cells plus 0.1–1 mg BCG were injected i.d. to the exterior side of upper arm of the patient on day 1–2 after the operation. The remaining tumor cells were kept in a liquid nitrogen bomb,

and the same treatment was performed repeatedly at 2, 4, and 8 postoperative weeks according to the harvested tumor cell number. Mitomycin C, 5-FU and futraful were given as anticancer agents, and in some cases PS-K or ATSO was given in conjunction. Examination as shown in Table 2 was conducted periodically. No side effects were noted, except for a shallow ulcer persisting for 2–3 weeks on the injection site. Table 3 shows ten cases of gastric cancer and three

TABLE 2. CLINICAL AND IMMUNOLOGICAL EXAMINATIONS CONDUCTED ON PATIENTS OF THE STUDY

Clinical examinations	Immunological examinations
Hematological examinations	Skin tests
Classification of white blood cells	PPD reaction
Thrombocytes	DNCB reaction
Urinary examination	Lymphocyte blastformation rate
Liver function	T and B-cell ratio
Serum protein	Macrophage migration inhibitory test
Chest X-ray	Lymphocytotoxicity test
Scintigram	Immunoglobulins
Lymphangiography	

Clinical and immunological examinations were performed periodically during the follow-up period in all cases receiving immunotherapy.

TABLE 3. PATIENTS TREATED BY BCG IMMUNOTHERAPY

No.	Age	Sex	Stage	BCG	PPD reaction	Survival (months)		Present status
1	75	M	III		+ → +	37		Alive
2	64	M	III		+ → +	13		dead of other disease
3	69	M	III		+ → −	28		Alive
4	69	M	III		− → ±	20	(liver metastasis)	Alive
5	46	F	IV, N_4P_1	+PSK	± → +	10	(cancerous peritonitis)	Dead
6	63	F	IV, P_1		+ → ±	39		Alive
7	70	M	IV, H_1	+PSK	+ → +	9	(cancerous peritonitis)	Dead
8	51	M	IV, P_3S_3		− → −	10	(cancerous peritonitis)	Dead
9	60	F	IV, P_3	+PSK	± → ± → +	16	(colon metastasis, ileus)	Dead
10	67	F	IV, P_2	+PSK	+	7	(icterus)	Dead
11	58	M	D-C, H_3		+ → + → ++	25	(liver metastasis)	Alive
12	67	M	D-B,		+ → +	4	(renal failure)	Dead
13	70	M	D-C, H_3		− → +	21		Alive

Ten patients were operated on for gastric cancer and 3 patients for colon cancer. All patients received BCG plus autochthonous tumor cell injections at least 3 times. The stage of cancer was III or IV in gastric cancer and Dukes C or B (D-C or D-B) in colon cancer. In some cases PSK, a kind of polysaccharide extracted from Coriolus Vesicolor Quel, was used.

PPD skin tests were measured before the operation and after the termination of immunotherapy.

cases of colon cancer treated by BCG plus autochthonous tumor cell injection at least 3 times. Gastric cancer in Stage IV represents cases where either P or H is positive. Case 6 has P_1 and is still surviving after a lapse of 39 months, and case 13 has H_3 of Dukes C but is now positive to the tuberculin test and surviving at 21 months after the operation. Case 9 is in Stage IV of $N_3S_2H_0P_3$, and only the main lesion is extirpated. This patient received immunization 4 times and also a daily administration of PS-K (6g/day). MIF was weakly positive, both the T-cell count and PHA-blastformation rate improved for about 6 postoperative months, but the MIF disappeared, and T cell count as well as blastformation rate showed a decreasing tendency in the tenth month, so that BCG alone was injected. MIF again increased, and ileus occurred at 1.3 postoperative years. Laparotomy was performed but the P_3 observed at the first operation had practically disappeared. In reviewing the survival of 6 cases of Stage IV gastric cancer patients immunized with a mixture of BCG and tumor cells, 8 cases of PS-K group receiving over 720g of PS-K (6g/day) and i.v. injection of 4mg mitomycin C twice weekly and 17 cases undergoing operation in the same period, the 6-month survival ratios were 6/6, 7/8, and 15/17 cases, respectively, and the 12-month survival ratios were 2/6, 3/8 and 8/17 cases, respectively. Stage III patients receiving BCG plus tumor immunization all survived for 12 months. On checking changes in the tuberculin test before, and 4 weeks after operation, those who turned positive after operation were: BCG group 3/9 cases, PS-K group 4/13, and the operation-alone group was 2/26. The group immunized with BCG showed the highest positive rate. MIF was reinforced in the BCG plus tumor-immunization group where MIF activity tended to persist for a long period of time (Fig. 5). The MIF turned negative in 8 of 11 cases prior to clinical confirmation of reccurrence. From the above findings, it seems necessary to conduct immunotherapy more intensively over a longer period of time.

Since 1975 we have been using BCG-CWS that has a stronger immunopotentiating power, a uniform chemical property and no danger of infection. We generally administer mitomycin C, 5-FU and futraful periodically after the operation, PS-K or ATSO as an immunopotentiator daily, and BCG-CWS alone once a week for 4 consecutive weeks and after 6 months once monthly for two years. The injection of BCG-CWS is continued for two years. In addition, on the basis of our experimental data that BCG injection into the tumor one week prior to tumor extirpation prolongs the survival of mice, 100–400 mcg BCG-CWS in divided doses were injected into the tumor tissues under endoscopy 7–10 days before the operation of gastric cancer and rectal cancer. Ever since Morton's report (37) of viable BCG injection into melanoma resulting in shrinkage of the tumor, many reports have appeared. According to statistics by Zbar *et al.* (38), melanoma shrinkage was seen in 58% (72/125) of cases receiving BCG intra-

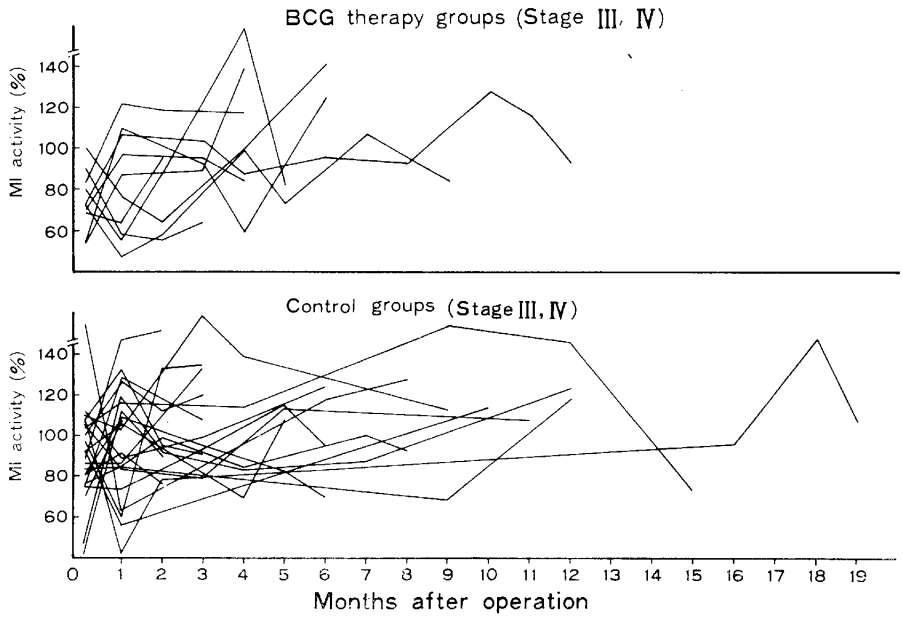

Fig. 5. MI activity after operation for stomach cancer. The MIF activity of the BCG immunotherapy group tended to continue positive longer than that of the control group without immunotherapy for at least several months after the operation.

tumor injection and in 14% (15/110) of cases not receiving BCG. Similar effects were observed in cases of mammary cancer and metastatic foci to skin. It is said that such treatment is effective only in cases positive to the tuberculin test and that there is marked infiltration of histiocytes and small round cells. We also observed redness and edema macroscopically and marked round cell infiltration, the appearance of Langhans giant cells and cytolysis of cancer cells microscopically at the BCG-CWS injection site of extirpated tumor specimens of our tuberculin-positive cases. Similarly marked swelling and enlargement was observed in regional lymph nodes.

At early stages of cancer, splenectomy seems to produce anticancer activity in the tumor-bearing host. When splenectomy is performed 5 days after s.c. transplantatation of 5×10^6 Ehrlich cancer cells to the back of mouse, the proliferating tumor began to shrink about 20 days later, and 85% of established tumors had completely regressed 35 to 50 days later, while control mice with no splenectomy died of tumor effects within 30–50 days. However, splenectomy tended to result in earlier death if it was performed on day 10 after transplantation. The survival rate of 89 gastric cancer cases on whom splenectomy was combined with tumor removal at our surgical department and related hospitals during the period 1965–1969 and in 400 non-splenectomized cases in the same period showed that

the 5-year survival rate was 20.2% in the splenectomized group and 48.6% in the non-splenectomized group. By stages, the survival rate was 100% (2 cases) in Stage I and 88.9% (9/11 cases) in Stage II of the splenectomized group, while it was 87.1% and 77.1% in the non-splenectomized group, but in cases of Stages III and IV the prognosis was poorer in the splenectomized group. There are many problems yet to be solved before splenectomy is routinely combined with immunotherapy, but heightening of host anticancer activity appears possible in the future by such combined therapeutic procedures.

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