



Title	Treatment of gastric cancer with HUD, an antigenic substance obtained from patient's urine
Author(s)	添田, 百枝
Citation	日本医学放射線学会雑誌. 1968, 28(9), p. 1265-1278
Version Type	VoR
URL	<a href="https://hdl.handle.net/11094/19024">https://hdl.handle.net/11094/19024</a>
rights	
Note	

*The University of Osaka Institutional Knowledge Archive : OUKA*

<https://ir.library.osaka-u.ac.jp/>

The University of Osaka

## Treatment of Gastric Cancer with HUD, an Antigenic Substance obtained from Patient's Urine

Momoe Soeda

(II. Research Institute, Technical Research and Development H.Q., J.D.A.)

### 癌患者尿から得られた抗原物質, HUD による 胃癌の術後再発防止療法について

防衛庁技術研究本部第2研究所 主任研究官衛生研究室長

添 田 百 枝

(昭和43年3月1日受付)

胃癌の早期診断はここ数年、飛躍的改善をとげたとはいえ、各方面の統計で明らかのように、早期発見にもかかわらず、根治手術例中、生存率は15%にしか過ぎない。

著者は、1960年、Ehrlich ascites tumor の罹患マウスの腹水遠心上清液中に、マウスに腹水癌をおこさせる病原因子を見出し、しかも、この上清を、化学的に精製して、得られた抗原物質 E.A.D. (Ehrlich ascites tumor's derivative) が、抗腫瘍性を積極的に高めることを発見したので、この免疫強化法の、人体応用の道を癌患者の尿に求めたところ、EAD様物質として、HUD. (Human Urine's derivative) が発見された。

このものは、水溶性物質で、白色無晶形粉末

で、マウスに対する LD<sub>50</sub> (ip) は、5000mg/kg 以上でも、毒性はほとんど小である。

マウス、ラット、家兎に対し慢性毒性は認められない。

HUDの使用法：根治手術後、可及的速かに、あるいは、術前投与し、1日40～150mgを1—2回皮下または筋肉内注射を行なった。

患者の内容、胃癌8例、直腸癌2例、卵巣癌再発1例について、現在(1968, 10, 31)で、最初例は3年半を経過するが、再発を防止している。今後、遠隔成績を見守ると同時に、本物質の理化学的性状を明らかにし、抗原物質相互間の異同を明らかにしたい。

#### Introduction

Although the detection of the early lesion and its immediate resection are the best measures for treatment of gastric cancer, it is well known that the majority of patients thus cured are the carriers of a superficially spreading type of gastric cancer, which tends to grow along the layer of mucous membrane and not readily to invade the deeper layers of the stomach wall. On the other hand, the usual type belongs to a deep-penetrating group, which rapidly invades the deeper layers and easily develops metastases to lymph nodes and the other organs.<sup>1)</sup> Unfortunately, the detection of the early stage of the latter type is far more difficult than that of the former type in spite of the fact that the diagnostic technics for detection of early gastric cancer have remarkably advanced in recent 10 years. For this reason, the over-all detec-

tion rate of the early gastric cancer is very low even at present, so that the majority of even operable patients cannot be cured because of both too late detection of the primary lesions and unavailability of adequate measures for inhibition of postoperative recurrence of the disease. Although the insidious onset of the clinical manifestations may mainly contribute to such late detection, lack of simple and reliable technics for diagnosis of the early primary lesions may possibly play an important role in failure in the early detection of the disease.

In any way, the five-year-survival rate of all patients who can receive a radical operation of the primary tumor is still very low and rarely exceeds the level of 20 per cent<sup>3)</sup>.

Consequently it seems urgent to develop a certain effective means to inhibit metastasis or growth of cancer cells not cleared by operation. Of course, radiation therapy and anti-cancer chemotherapy have been extensively tested for many years on their effect to control such postoperative metastasis or growth of tumor cells, however, it may fairly be said that both measures have almost completely failed in this purpose up to the present time.

Both measures are known to be highly cytotoxic not only for malignant tumor cells but also for normal cells of host organs and tissues, especially those of hematopoietic and lymphatic tissues. Such side-effects are usually evidenced by development of abnormal findings such as lymphopenia, leukopenia, thrombocytopenia, reduction in hemoglobin content and hematocrit, dysfunction of the liver, and gastrointestinal disturbances.

The cells of lymphatic origin, the lymphocytes and plasma cells, are most sensitive to ionizing radiation or cytotoxic chemotherapeutic agents, as it is evident from the early development of a significant reduction in their count in the peripheral blood. Especially, the plasma cells are well known to be the site of antibody production and, in addition, these cells have been shown to be related to host resistance against gastric cancer.<sup>2)</sup> The reduction of the plasma cells due to radiation therapy or chemotherapy may presumably deteriorate host immunity against tumor and accelerate active growth of the cancer cells which have survived the deleterious effect of ionizing radiation or cytotoxic agents.

In our country, a great many patients with gastric cancer are postoperatively treated with various anti-cancer agents such as Mitomycin C, Endoxan and Toyomycin, but none of these agents could have contributed to a significant improvement in the survival rate of operated patients. Although there may be many other factors contributory to invariable failure of these agents, their deleterious effect on immunologic competence of host would provide one of the most disadvantageous conditions for control of cancer recurrence. For this reason, it seems reasonable to consider that an ideal anti-cancer agent in future should exert its inhibitory effect on malignant tumor cells without causing any deleterious changes in immunologic mechanism of patients.

In an attempt to find an effective means to inhibit postoperative recurrence and thus to achieve a significant improvement of the five-year-survival rate in patients with gastric cancer, experimental studies were performed on both preventive and curative measures for transplantable ascites tumors of animals, and we came to the conclusion that tumors should be controlled by reinforcement of host anti-tumor immunity, because it was experimentally demonstrated that the level of immunity naturally developed in tumor-bearing animals is too low to inhibit further growth of tumor, but it can artificially be reinforced to such a level as sufficient to cause damage to tumor cells.<sup>3)</sup> Immunologic resistance of mice against Ehrlich ascites tumor can be induced or reinforced by i.p. injection of not only inactivated tumor cells, but also the

cell-free supernatant of centrifuged ascites from tumor-bearing mice. Immunogenic substance responsible for induction of animal resistance against ascites tumor may possibly be present in such a cell-free inoculum. Pooled ascites specimens were centrifuged and the supernatant was subjected to a series of chemical procedures with the yield of a specific fraction which proved fairly effective to afford a significant protection to mice against i.p. implantation of the original tumor cells. This fraction was obtained in a freeze-dried form and named EAD (Soeda, 1961).<sup>6)</sup>

A variety of human specimens were tested and it was found that the urine of patients with cancerous disease almost invariably contains a considerable amount of a substance chemically similar to EAD, which was chemically extracted and named HUD (Soeda, 1961).<sup>9)</sup> The urinary content of HUD shows a great variety according to the conditions of tumor-bearing patients, and it usually shows a rapid reduction within one week after operation.

It was clinically applied to an intractable case of metastatic ovarian cancer in June, 1965, and we were much impressed with its excellent effect on regression of metastatic tumors. Almost all metastases completely disappeared during a course of 3 months after the start of HUD therapy, and the patient was discharged under a quite favorable condition. More than 30 months have passed since she was discharged and now she is completely well and enjoying the rest of her life.

Since March, 1965, HUD has been applied mainly to patients with gastric cancer for the purpose of prevention against postoperative recurrence of the disease.

The present report describes an outline of the results of clinical application of HUD to total 16 patients consisting of 8 radically operated cases of gastric cancer, 5 inoperable or metastatic cases of the same disease, 2 cases of rectal cancer and one case of ovarian cancer, admitted to Tokyo Teishin Hospital, Tokyo.

#### **Experimental observations in relation to clinical application of HUD**

##### **1. Artificial reinforcement of animal resistance against ascites tumors.**

The majority of mice of ddS strain cannot resist against an i.p. implantation of  $10^2$  Ehrlich tumor cells and die within 2 to 4 weeks as a result of typical ascites tumor. A level of  $10^8$  tumor cells kills almost all mice, so that it may be regarded as an absolutely fatal dose for ordinary mice of this strain. The transplatability of tumor cells is lost by keeping them at  $55^\circ\text{C}$  for 30 minutes, however, they still retain antigenic properties capable of inducing anti-tumor resistance in mice. Thus, a single i.p. inoculation of  $10^8$  inactivated tumor cells usually affords protection against a subsequent i.p. implantation of  $10^4$  intact tumor cells, an absolutely intolerable dose for usual animals. If the same vaccination with inactivated cells is repeated 3 or 4 times, once a week, about a half of animals are effectively immunized to such an extent as resistant against a challenge implant of  $10^6$  tumor cells.<sup>6)</sup> This is also true in the case of Yoshida sarcoma in rats or Sarcoma-180 in mice.

Besides, a similar enhancement of anti-tumor immunity of animals can be achieved by i.p. vaccination with an appropriate dose of the tumor-cell-free supernatant of ascites from tumor-bearing animals. Some antigenic agent responsible for induction of anti-tumor immunity in animals seems likely to be present in such cell-free ascites. Eventual effect of both vaccines is almost identical with respect to reinforcement of animal immunity, and thus the mouse can be made insusceptible to an inoculum of  $10^6$  Ehrlich ascites tumor and Sarcoma-180 cells, and the rat to an inoculum of  $10^7$  Yoshida sarcoma cells, but further potentiation of animal immunity can hardly be achieved by this procedure.<sup>6)7)</sup>

##### **2. Anti-tumor activity of the spleen cells of immunized animals**

#### a) Heterologous spleen cells

Rabbits are immunized by i.v. injection of cells of Ehrlich ascites tumor or Yoshida sarcoma and the spleens are removed to prepare spleen cell suspensions. A single i.p. injection of a dose of  $5 \times 10^8$  spleen cells of rabbits immunized against Ehrlich tumor cells proved capable of rescuing about 60% of mice from ascites tumor death following i.p. challenge with  $10^6$  Ehrlich ascites tumor cells, whereas more than 80% of rats similarly treated with immunized spleen cells against Yoshida sarcoma did not develop ascites tumor even when they were challenged with  $10^7$  cells of Yoshida sarcoma. A similar effect cannot be recognized with either non-immunized spleen cells or the sera of immunized rabbits, while the inhibitory activity of immunized spleen cells can readily be inactivated by application of ultrasonic waves, so that it seems likely that intact and immunized spleen cells may be essential for exhibition of such anti-tumor activity.<sup>7)</sup> Almost the same results were shown by Stuart's experiment with Landschutz ascites tumor of mice.<sup>4)</sup>

#### b) Homologous spleen cells

The spleen cells of mice or rats with manifest signs of ascites tumor, or those of mice or rats with experimentally reinforced immunity also have a similar anti-tumor activity. Thus, a single i.p. injection of  $5 \times 10^8$  spleen cells of such mice can prevent development of Ehrlich ascites tumor in about 60% of mice following i.p. implantation of  $10^4$  tumor cells, while a similar procedure can rescue about 80% of rats from death of ascites tumor due to challenge with  $10^6$  Yoshida sarcoma cells.<sup>8)</sup>

In contrast with heterologous spleen cells, the anti-tumor activity of homologous spleen cells seems to be considerably inferior, however, in view of the facts that it is also readily inactivated by exposure to ultrasonic waves and that the sera of immune animals or spleen cells of normal animals do not show such activity, it seems likely that there exists no fundamental difference in the mechanism by which both types of cells exert their anti-tumor activity.

Stuart has suggested that anti-tumor activity of spleen cells of immunized rabbits may be due to antibody carried by them on their surface of interior which permits them to adhere to tumor cells to cause their destruction.<sup>4)</sup> According to this concept, anti-tumor activity of immunized spleen cells from homologous animals may also be due to antibody carried by them, and reinforcement of anti-tumor immunity of susceptible animals induced by vaccination may be due to enhanced immunological competence of host to elaborate antibody-carrying cells in the lymphatic organs and tissues.<sup>8)</sup>

#### 3) Significance of EAD for reinforcement of anti-tumor immunity

In 1961, Soeda and Sumiyama reported on the following experimental findings. Ascites was collected from mice with typical Ehrlich ascites tumor and centrifuged at 4,000 to 20,000 rpm for 30 minutes to obtain cell-free ascites fluid. An amount of this fluid corresponding to  $10^4$  tumor cells was shown enough for induction of typical ascites tumor in the majority of ddS strain mice, when it was i.p. inoculated into animals.<sup>5)</sup> For this reason, it seems highly probable that a certain agent capable of inducing malignant transformation of host cells may exist in such a tumor-cell-free ascites fluid. Ascites tumor thus induced was demonstrated to be serially transplantable with ease, and by cross-challenge experiments it was shown antigenically identical with the original ascites tumor.

A dose of cell-free ascites which corresponds to  $10^2$  or less could not induce ascites tumor in animals, but such animals became, within one week, insusceptible to a subsequent implantation of  $10^8$  Ehrlich tumor cells, an absolutely fatal dose for normal mice. This means that a sublethal dose of cell-free ascites given to animals can effectively induce a significant level of anti-tumor immunity without provoking any signs

of ascites tumor. By repeated inoculation of this dose, the level of such immunity can gradually be elevated and the animals may eventually become resistant against i.p. implantation of a dose of  $10^6$  tumor cells.

Ascites specimens were collected from mice with manifest signs of ascites tumor and subjected to centrifugation at 4,000 rpm for 30 minutes, and the supernatant was chemically treated to extract an effective fraction to induce animal immunity against the original ascites tumor (EAD). Of course, it does not provoke ascites tumors in animals, but it still retains its capacity to induce or reinforce anti-tumor immunity when it is parenterally injected into animals. Three successive injections of a dose of 0.5 mg EAD, once a week, is usually enough to afford protection to animals against i.p. implantation of a dose level of  $10^6$  tumor cells.

Thus, animal immunity provoked by chemical preparation of cell-free ascites like EAD can serve specifically to resist the onset of ascites tumor even in highly susceptible animals. Such anti-tumor resistance, therefore, may not necessarily be due to antibody against tumor cell itself, but it may rather depend upon a certain antibody produced in response to some agent existing in cell-free ascites, which is responsible for malignant transformation of host cells as mentioned before. If this agent is inactivated by its specific antibody, at least its activity to induce malignant transformation will be completely inhibited.

On the other hand, spleen cells of mice immunized with EAD were shown to have a beneficial inhibitory effect upon i.p. implanted Ehrlich tumor cells. This may indicate that such spleen cells may carry the same antibody as that of spleen cells from mice immunized with tumor-cell-vaccines.

Taking these facts into consideration, it seems reasonable to consider that the identical principle with regard to immunization of animals may be contained in both cellular and non-cellular vaccines, which stimulates host to produce a specific antibody against itself when inoculated into animals. Such antibody may serve not only to destroy tumor cells, but also to inhibit malignant transformation of normal host cells. Consequently, tumor cell itself is not necessarily required as an useful antigen for induction of antitumor immunity or for its reinforcement in animals.

In any way, these facts may be expected to implicate some important possibilities in practical application of this principle to human cases. EAD is almost non-toxic for animals and can repeatedly be injected without any danger of injurious side-effects. It can be maintained for fairly a long time in a stable freeze-dried form and is readily soluble into saline or distilled water. Similar preparation was obtained from cell-free ascites collected from rats with manifest symptoms of Yoshida sarcoma, which proved capable of inducing a significant degree of immunity against corresponding tumor in rats. This was also proved to be the case in Sarcoma-180. Thus, it became clear that although anti-tumor immunity naturally provoked in highly susceptible animals in response to their own tumors may be negligible to counteract tumor growth, it may artificially be reinforced up to a significant level by vaccination with chemical preparation like EAD.

#### 4) Therapeutic experiments with Ehrlich solid tumor

To test EAD on its therapeutic effect on tumor growth, a dose of  $10^4$  Ehrlich ascites tumor cells each was s.c. inoculated into mice to produce solid tumors. In normal mice such tumors rapidly grow and kill almost all animals within 4 to 8 weeks after tumor cell implantation. In contrast, if repeated s.c. injection of 0.5 mg EAD each is started within 5 days after tumor cell implantation, the growth of developed tumor may be suppressed or slowed on the way, and after it has enlarged to a certain size during a course between 2 to 4 weeks after tumor cell implantation it begins to regress and finally disappears or is extruded from the

skin. Reimplantation of  $10^4$  tumor cells at the site where a solid tumor has once regressed invariably fails to develop a tumor. Reimplantation at the other sites may result information of small solid tumors, however, they usually regress spontaneously within one week. Similar results were obtained with tumor-cell-vaccine as noted above.

If a dose of 0.5 mg EAD each is s.c. inoculated at a random site of the animal skin, 3 to 5 times every third day, the site may become increasingly resistant until it is completely immune against local implantation of intact tumor cells. At this stage, almost every spot of the skin acquires a higher resistance which does not allow ready growth of a subcutaneous solid tumor. In general, such animals are also resistant against i.p. implantation of a dose level of  $10^4$  tumor cells.

Thus, even growing tumors can be suppressed by repeated injection of proper vaccines if it is started within the stage when tumor cell masses are not so large. Even in advanced stage of solid tumors repeated injection of EAD is sometimes successful in complete demarcation of a larger tumor until it is extruded from surrounding normal tissues. Such animals were reimplanted with  $10^4$  tumor cells at the site of healed scar 6 months thereafter, but no tumor developed at all. This may indicate that local immunity thus induced is not of temporary character, but it may last for fairly a long period. It is of much interest that anti-tumor immunity is first induced locally at the site of vaccination, and then it gradually spreads until it becomes more or less systemic. This fact may support the view that anti-tumor immunity may mainly depend upon cellular antibody, not upon humoral antibody. There seems to exist no fundamental difference in the mechanism by which both prophylactic and therapeutic effects of cellular or non-cellular vaccine can be exerted. Reinforcement of anti-tumor immunity due to EAD may be responsible for its beneficial therapeutic activity against tumor.

5) Clinically applicable implications of above experimental findings

a) The reinforcement of animal immunity against ascites tumor was achieved by repeated inoculation of non-cellular vaccines like EAD or YSD. The antigenic activity of such vaccines was shown to be identical with that of cellular vaccines, and both types of vaccine produced almost the same effect in animals.

b) Natural anti-tumor immunity induced in susceptible animals is almost negligible in relation to inhibition of the growth of their tumors, but if it is reinforced by the use of proper vaccines, it may sufficiently serve to interrupt or to suppress the growth of their tumors. This fact may indicate an important role of anti-tumor immunity in control of tumor growth even in highly susceptible animals. As a result, host immunity against tumor should be reinforced as high as possible to eliminate the source of malignant tumors.

c) It seems certain that there exists a certain upper limit in the extent of artificial reinforcement of anti-tumor immunity of animals, so that it seems better to start vaccination during the early stage of tumor growth, or within the stage when the number of tumor cells is still not so large.

According to these implications, it seems reasonable to consider that whatever anti-cancer therapeutic means we select, we should not apply any measures which may cause an injurious effect on immunological mechanism of host against cancer. Even if such measures may apparently induce a dramatic therapeutic effect on cancer lesions, their cytotoxic effect on host cells with capacity to produce antibody may simultaneously lower the host resistance which leads to an active growth of cancer cells remained resistant against the damage due to their anti-cancer activity. This will probably explain the mechanism of eventual

failures in postoperative treatment with X-ray irradiation or various anti-tumor drugs. Host immunity against cancer should, therefore, be rather reinforced as far as possible, especially within the early stage of growth or immediately after total resection of the primary tumors.

In an attempt to obtain a proper immunogenic substance for reinforcement of human anticancer immunity, various human specimens were subjected to a series of chemical procedures to extract a substance similar to EAD obtained from mice ascites.

First, specimens of cancer tissue removed by operation were homogenized and extracted with saline, which was then centrifuged at 3,000 rpm for 30 minutes. The supernatant was extracted with almost the same chemical procedures as those applied to mice ascites. Second, the supernatant of peritoneal exudate accompanying peritonitis carcinomatosa was also employed for this purpose, but both specimens were shown inadequate to yield an EAD-like substance because of a high content of protein and other unrelated impurities which caused many difficulties in performance of chemical treatment.

On the other hand, the urine of patients with cancerous disease was demonstrated to contain a significant amount of EAD-like substance readily extractable with chemical techniques as used for animal specimens. This was named HUD (1961). The urinary content of HUD is usually maintained at a higher level in patients with overt cancer, but it rapidly reduces to an almost negligible level within one week after total resection of the primary tumor. On postoperative recurrence, its content increases again and reaches a high level in the later stage of the disease. In view of these facts, it seems likely that HUD may be related to cancer tissues within the body or their cellular constituents.

It is almost non-toxic for animals and its i.p. LD<sub>50</sub> for individual mice of ddS strain is approximately 2,000 mg/Kg. Intraperitoneal injection of a dose of 1.0 mg HUD into individual mice, 3 times every third day, usually causes the enlargement of the spleen with an increase of the cell count to a level 2 to 3 times as high as the normal range of level, which well resembles the picture in mice similarly treated with EAD.

#### 6) Clinical application of HUD

HUD was first applied to fairly advanced, inoperable cases of gastric and ovarian cancer to test on it activity to induce reinforcement of human anticancer immunity. Of course, it was very difficult to reasonably appreciate its effectiveness in these intractable cases, however, in 1965 it was clearly demonstrated in a patient with ovarian cancer that a sufficient degree of reinforcement of anticancer immunity can be achieved by repeated injection of HUD.

A female, 67 years of age, was diagnosed as affected by a malignant tumor of the left ovary on May 3rd, 1963. Surgical resection of the affected ovary was immediately performed, but radical resection was not feasible because of invasive lesions in the surrounding tissues and of lymph node metastases. The primary tumor was histologically diagnosed as "Adenocarcinoma ovarii", and the operation was followed by Co-60 irradiation with total 5000 r in dose. About 3 months after operation a tumor developed under the abdominal skin near the navel, which was resected and histologically proved to be due to metastasis from the original ovarian carcinoma. The local area was again irradiated with Co-60, 7200 r in dose, but about 4 months later another metastatic tumor developed under the abdominal skin near the first metastatic tumor, and since then similar tumors appeared, one after another, in the wide regions of the abdomen. In spite of the third trial of Co-60 therapy, the abdominal wall was more and more indurated to reveal an armor-like appearance. Then metastasis occurred to the right axillary lymph node which enlarged up to the size of a pigeon's egg. At this stage, HUD therapy was started. Daily administration of 100 mg

of HUD, prepared from patient's urine, was done for 91 consecutive days, by which the tumors in the right axilla began to regress about 40 days after the start of therapy and almost completely disappeared by the end of therapy. Almost in parallel with this, the indurated abdominal wall began to soften and returned to normal during a period of 4 months. Besides these, a remarkable tendency to develop frequent metastases which had never been controlled by both radiological and surgical measures was completely subsided after the start of HUD therapy. About 33 months have passed now since HUD therapy was initiated, and she is now completely well and enjoying the rest of her life. The patient is now 72 years old.

By this clinical course, it was clearly shown that repeated administration of HUD being capable of inducing elevation of human anticancer immunity to such a level as sufficient to cause damage to their own cancer cells. Complete control by HUD therapy of a remarkable tendency to develop metastasis may strongly suggest that sufficient immunity for suppression of tumor growth has been established by this method and maintained for fairly a long period. These results are regarded as almost identical with those of EAD therapy of s.c. solid tumors produced in mice by s.c. implantation of Ehrlich ascites tumor cells. It is also evident from this human case that anticancer immunity naturally induced in tumor-bearing patients seems almost negligible in respect to suppression of their own tumors, but it may artificially be reinforced up to a level which is sufficient not only for inhibition of tumor growth, but also for destruction of actively growing cancer cells.

In spite of such an excellent response in this ovarian cancer, HUD therapy could not induce an inhibitory effect upon actively growing cells in inoperable or recurrent cases of gastric cancer. This failure may be due to an unbalance between the level of host immunity attainable by HUD therapy and the magnitude of cancer cell population in such advanced cases. In animal experiments, it was also shown that there exists a certain upper limit in artificial reinforcement of animal immunity against tumor, which is almost impossible to overcome. For this reason, it is reasonable to start HUD therapy as early as possible while the number of cancer cells is still small or immediately after total resection of the primary tumors. This is the reason why we have tested HUD on its control effect upon postoperative recurrence of gastric cancer.

#### **Clinical Results of HUD Therapy**

As noted above, HUD therapy may be expected to exhibit its most beneficial activity when the number of cancer cells is fairly small. The early stage of gastric cancer may certainly provide one of such circumstances, but its postoperative prognosis is well known to be far more favorable than that of more advanced stages, and it does not need any additional treatment after operation. Besides this, a period immediately after total resection of the primary cancer lesion may provide another suitable condition for commencement of HUD therapy, because the majority of cancer cells are expected to be removed by operation. Except for that of rare cases of early gastric cancer, the postoperative prognosis of patients, whose primary lesion have been totally resected, is usually very poor because of an unusually high rate of recurrent attack of the disease. It may fairly be said that at present more than 80 per cent of even operable patients with gastric cancer cannot be cured because of unavailability of suitable measures for complete suppression of postoperative recurrence originating from a small population of cancer cells remained after operation. Consequently, if HUD therapy is effective in suppressing such recurrence, it would provide one of the most promising measures remarkably to improve the curability of patients with at least operable gastric cancer.

As it is evident from the above case of ovarian cancer, a satisfactory reinforcement of anticancer immu-

nity may possibly be induced in cases of gastric cancer, however, a fairly long period may be required until such a level of immunity can be achieved. HUD therapy, therefore, should be started immediately after operation and continued for at least 40 days. However, from practical standpoint, it is difficult to continue this therapy for more than one month, because patients usually do not stay in hospital so long after operation.

I presented here an outline of the clinical results of a group of 8 patients whose primary gastric lesions were radically operated, and of another group of 8 patients consisted of 5 cases of inoperable gastric cancer, 2 cases of operated rectal cancer and one case of ovarian cancer with metastasis. Among 5 cases of gastric cancer there were 3 cases whose lesions were subtotally resected, while the other 2 cases were entirely inoperable. Two cases of rectal cancer were treated with Co-60 irradiation following subtotal resection of the primary lesions. The clinical course of the patient with ovarian cancer was already mentioned in the previous paragraph.

#### Patients with gastric cancer, in whom the primary lesions were totally resected

Total 8 cases were treated with auto-HUD starting immediately after operation. Except for No. 8 case, HUD was prepared from the urine collected shortly before operation, and its daily dose of 60 to 150 mg was i.m. injected for 26 to 48 days. According to Borrmann's classification, 5 cases were typed as III. grade, 2 cases as II. grade, while only one case was of early stage. On operation lymph node metastases were recognized in 7 out of 8 cases (Table 1).

Table 1. Patients with gastric cancer, whose primary lesions were radically operated

No. of patients	1.	2.	3.	4.	5.	6.	7.	8.
Patient	A.S.	T.T.	S.S.	T.A.	S.T.	T.I.	T.A.	H.N.
Sex	Male	Female	Female	Female	Female	Female	Male	Male
Age	25	63	41	46	58	59	61	59
Date of operation	1,3, 1965	10,3, 1965	30,3, 1965	22,4, 1965	4,5, 1965	14,5, 1965	3,6, 1965	25,6, 1965
Size of the primary lesion, the longer diameter in cm	more than 8	2	6-7	2-3	7	2-3	2	4-5
Lymph node metastasis	Pos.	Neg.	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.
Borrmann's type of	III	I	III	II	III	III	III	II
Period from operation to date, in month	36*	35	35	34	34	33	33	Died after 24
Alive or dead	Alive	Alive	Alive	Alive	Alive	Alive	Alive	Dead
Recurrence	No	No	No	No	No	No	No	Yes
Metastasis	No	No	No	No	No	No	No	Yes
Total dose of HUD, in gm	2.33	7.12	2.20	1.63	2.28	2.80	2.10	1.20
Daily dose of HUD, in mg	90	150	60-70	60-70	50-70	60-80	60-70	40

\*The above table indicates the states as of February 20th, 1968.

As shown in Table 1, a case (No. 8) developed recurrence about 20 months after operation and died about 4 months thereafter.

This was an exceptional case, because his HUD was prepared from his postoperative urine with an

insufficient yield for therapeutic purpose. Thus only 1.2 gm of HUD was used for this case, which was the least in our series.

With an exception of No. 8 case, all of 7 cases have been completely well for 33 to 36 months after operation and now they are participating in their almost full activities without any signs of recurrence.

According to Yamagata's statistical review, the 3-year-survival rate of operated patients with gastric cancer whose primary lesions had invaded the serous membrane of the stomach wall with lymph node metastasis was 40.4 per cent (84/208), which means that more than half were dead within 3 years.<sup>3)</sup> Although the number of patients is too small, the fact that all of 5 cases in our series, whose lesions were typed as Borrmann's III type with lymph node metastasis, have survived for more than 33 months with no signs of recurrence may indicate that at least we cannot ignore a certain beneficial effect of HUD on inhibition of postoperative recurrence of gastric cancer.

I have presented here only 8 cases, in which more than 33 months have passed since operation was performed. Besides these cases, however, more than 10 cases have been similarly treated with similar beneficial results, but only 16 to 22 months have passed now since operation was performed. With regard to the presented cases, careful follow-up study should be continued for further several years, however, it seems likely from their clinical results up to date that HUD therapy may also induce reinforcement of host immunity even in the cases of gastric cancer, and that if it is started immediately after total resection of the primary gastric lesions, anticancer immunity sufficiently to inhibit an active growth of remained cancer cells may be established in host and thus suppress the occurrence of postoperative recidiv of the disease.

For these cases no other therapy was done after operation. In particular, radiation therapy or medication of anticancer drugs was avoided as contraindicated, taking their injurious effects on host immunity into consideration. Consequently, it may fairly be said that the favorable results in this series may exclusively be due to the effect of HUD therapy.

#### **Patients with rectal or ovarian cancer**

Only 2 cases of rectal cancer and one case of ovarian cancer were post-operatively treated with HUD. With regard to the case of ovarian cancer, a brief description of her clinical course following operation was previously given in the paragraph of "Clinical application of HUD". By this instance it was clearly shown that HUD certainly has an activity to reinforce human anticancer immunity from a negligible level to a satisfactorily high level to induce damage of their growing tumors. It was also shown that such reinforced immunity may be maintained for fairly a long period, since spontaneous regression of metastatic tumors still went on after completion of HUD therapy, and no more metastasis have occurred for past 33 months.

With regard to rectal cancer cases, both were fairly advanced cases with lymph node involvement, and in addition they were treated with Co-60 irradiation not only immediately after operation, but also periodically for more than one year. One patient is now completely well and enjoying his retired life, but another case developed metastasis to the brain about 22 months after operation and died shortly thereafter. About 34 months have passed now since the former case was operated, without showing any signs of metastasis or recurrence of the disease (Table 2).

#### **Patients with gastric cancer, inoperable, recurrent or subtotally resected**

Of course, these cases did not appear, from the beginning, to be suitable for HUD therapy. Among these cases, there were 2 cases in which recidiv had occurred about 16 months after total resection of the

Table 2. Patients with inoperable or recurrent gastric cancer and those with rectal or ovarian cancer

No. of patients	9.	10.	11.	12.	13.	14.	15.	16.
Patients	O.H.	T.T.	M.M.	E.E.	S.N.	M.M.	F.K.	K.A.
Sex	Male	Male	Female	Male	Female	Female	Male	Male
Age	65	66	68	63	54	33	66	34
Diagnosis	Rectal cancer	Rectal cancer	Meta-static case of ovarian cancer	Meta-static case of gastric cancer	Recur-rent case of gastric cancer	Recur-rent case of gastric cancer	Inoper-able case of gastric cancer	Inoper-able case of gastric cancer
Date of operation	15,4, 1965	30,4, 1965	3,5 1963	29,5, 1964	13,11, 1963	6,11 1963	Inoper-able	Inoper-able
Operation	Subto-tal	Subto-tal	Total	Subto-tal	Total	Total		
Lymph node meta-stasis on operation	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.
Period from operation to date, in month	34	Died after 24	57	Died after 9	Died after 24	Died after 19		
Period between operation and onset of meta-stasis, in month		20	4	Already present	16	16	Already present	Already present
Recurrence after HUD therapy	No	Yes	No	Already present	Already present	Already present		
Alive or dead	Alive	Dead	Alive	Dead	Dead	Dead	Dead	Dead
Total dose of HUD, in gm	6.64	5.00	9.12	5.59	4.82	4.41	5.70	3.40
Daily dose of HUD, in mg	70-90	60-70	100	140	50-60	80-90	150-160	150-170

primary gastric lesion. One patient was a female, 54 years of age. About 16 months after operation she became aware of gradual swelling of the abdomen which was accompanied by frequent vomiting and difficulty in dietary passage. One month later she was again admitted and treated with i.m. administration of a daily dose of 50 mg of HUD, twice a week, for 16 weeks. By this therapy abdominal swelling much reduced, vomiting was gradually subsided and the dietary passage became much easier, so the patient was discharged, but 3 months later the abdomen again began to swell and her general weakness increasingly advanced and died shortly thereafter (Table 2).

Another case was a female, 33 years of age. Abdominal swelling began to occur about 16 months after total resection of the primary gastric lesion. She was admitted to the hospital and treated with daily administration of a dose of 90 mg HUD for 46 days, but her clinical condition did not appear to be appreciably improved and she died 4 months after the onset of abdominal swelling (Table 2).

Besides these cases, there were 2 inoperable cases of gastric cancer. When they were admitted to the hospital, their lesions had far advanced and metastasis was recognized in the wide range of the abdomen. HUD therapy was started at once, but almost no effect was recognized (Table 2).

There was another case in which subtotal resection was indicated. Metastasis had occurred to the wide range of lymph nodes and the mesenterium, peritoneum and liver had been disseminated by metastatic tumors when operation was performed. About 2 months after operation HUD therapy was started and a daily dose of 140 mg HUD was given, twice a week, for 2 months, but almost no beneficial effect was recognized (Table 2).

Thus, HUD therapy does not seem to exert a beneficial inhibitory effect upon inoperable or recurrent cancer growth. In some cases, however, an apparent improvement of clinical manifestations was temporarily observed in the course of HUD therapy, so that more early start and more long continuation of HUD therapy may probably be necessary to achieve a better result in these advanced gastric cancer. In any way, this failure may be due to difficulty in reinforcement of host immunity to such an extent as enough for destruction of so many cancer cells growing within these advanced cases.

### Discussion and Summary

In order to improve the curability of patients with gastric cancer, it is extremely urgent to develop an effective measure to eradicate remaining cancer cells following operation and thus to avoid the danger of postoperative recurrence of the disease. Of course, radiation therapy and anticancer chemotherapy have been extensively tested on such effectiveness, but it may fairly be said that both measures have failed to gain this purpose. Both measures are well known to be highly toxic for man, so their clinical application should be controlled by certain strict criteria. The anti-tumor effects of both measures are by no means exerted on cancer cells alone, but they cause more or less severe damage to host organs and tissues which results in development of a variety of undesirable side-effects. In particular, immunological competence of host may be severely injured by either of both measures, because the plasma cells which are known to be the site of antibody production are most sensitive to deleterious effect of both measures.

With regard to an important role of plasma cells in human anticancer resistance, the report of Urabe et al.<sup>2)</sup> may deserve a special attention. They have studied on a correlation between histological characteristics of the primary lesions of gastric cancer and the 5-year-survival rate of patients after operation, and reported that the 5-year-survival rate of hosts, whose specimens had revealed the histological characteristics that the plasma cells were predominantly found among interstitial cell elements and the affected areas by cancer cell invasion were entirely surrounded by numerous plasma cells, was more than 5 times as high as that of hosts without such histological pictures. On the other hand, predominant infiltration of lymphocytes or neutrophils into interstitial spacings was shown to bear no favorable relation to the survival rate of hosts.

Thus in total 30 cases, whose cancer specimens had revealed a complete enclosure of the affected areas by numerous plasma cells, the 5-year-survival rate was 66.7 per cent, whereas in total 40 cases without such histological pictures it was only 13.3 per cent. Besides these, there were 28 cases whose specimens had revealed an intermediate histological pictures, namely the affected areas had been only incompletely or loosely surrounded by not so many plasma cells. The 5-year-survival rate of these cases was 24.3 per cent.

In view of these facts, it seems likely that the postoperative prognosis of patients may depend upon their immunological competence to produce and mobilize immunized plasma cells around the sites where cancer growths occur, and the higher the competence of hosts the more plasma cells with antibody may be produced which permits a ready enclosure of invasive lesions with numerous plasma cells.

Such possibility was also suggested by experimental findings with animal transplantable ascites tumors. Spleen cell suspension of the rabbit immunized with tumor cells contains numerous lymphoid cells which being supposed to carry anti-tumor antibody on their surface or interior. Intraperitoneal inoculation of this suspension into animals proved capable of inhibiting the development of ascites tumors due to i.p.

implantation of ascites tumor cells. Homologous spleen cells also proved to be similarly effective to counteract the development of ascites tumors. These facts may suggest that even in highly susceptible animals they contract an active growth of ascites tumors with their immunological competence to produce immunized lymphoid cells and mobilize them to the sites where tumor growth occurs. If we experimentally stimulate immunological mechanism of animals with proper antigens, their capacity to counteract tumor growth may be reinforced to such a level as sufficient to survive i.p. inoculation of a tumor cell dose intolerable for untreated animals. This was experimentally demonstrated in mice immunized with EAD. The animals were shown to become insusceptible to i.p. implantation of an amount of tumor cells  $10^3$  to  $10^4$  times as large as a minimal transplantable dose for untreated animals. If it is also true in the case of human cancer, the immunological competence of patients should be considered as essential for natural defence force of host against cancer and, with every effort, it should be reinforced as far as possible by suitable measures to counteract cancer cell invasion.

HUD therapy was devised and tested along this line, and its effectiveness to induce such reinforcement of human immunity was clinically demonstrated in an intractable case of ovarian cancer as described above. Whether it also proves to be the case in gastric cancer is not clear at present, but if it proves to be the case, it would provide one of the most reasonable measures at least for postoperative treatment of gastric cancer, because the magnitude of cancer cell population may possibly be very small at this stage. Thus, HUD therapy has been tested mainly on its inhibitory effect on postoperative recidiv in patients whose primary lesions had been radically operated. During a period between March and June, 1965, total 8 patients were treated with HUD immediately after operation. In 5 cases in this group, the primary lesions were found to invade the serous membrane of the stomach wall and to be accompanied by lymph node involvement when operation was performed. In general, the postoperative prognosis of this group of patients has been very poor, and the 3-year-survival rate has been referred to as low as less than 40 per cent. About 3 years have passed now since the first case was operated, and 7 out of 8 patients are completely well and participating in almost full activities without any signs of recurrence. In particular, 5 cases described above have been quite well for about 3 years to date and even now they are completely free from any signs indicating recurrence of the disease.

In view of these facts, it seems likely that HUD therapy may be apparently effective to suppress postoperative recurrence of gastric cancer and that such effect may presumably be due to its antigenic property to induce reinforcement of host immunity against cancer.

Although follow-up studies of these 7 cases should be continued for further several years, the results obtained up to date may be, at least, superior to those achieved by postoperative therapy with radiation or anticancer drugs.

On the other hand, HUD therapy has failed in treatment of inoperable or recurrent cases of gastric cancer, the probable reasons for which were discussed before. In any way, the reinforced immunity by HUD therapy appears to be incapable of getting to a level which sufficiently gives rise to destruction of so many cancer cells growing in such patients.

This is an intermediate report on the results of clinical application of HUD to total 16 patients. Survived patients should be periodically examined for further several years, on which will be reported some other day.

### Acknowledgement

The author wishes to thank Dr. K. Kamiko, Director of II. Research Institute, J.D.A., for his interest and aid in this work, and also Dr. T. Kitahara, Chief of the Surgical Division of Tokyo Teishin Hospital, Tokyo, for his kind cooperation in clinical test of HUD.

### References

- 1) Inokuchi K. and Furusawa M.: Jap. Med. J., No. 2211, 3-9, 1965.
  - 2) Urabe M., Yamamoto K., Takano R., Takemori S. and Miura S.: Relation of the Interstitial Reaction of Gastric Cancer to the Follow-up Results after the Radical Operation. Jap. J. Cancer Clinics, 10: 791-799, 1964.
  - 3) Yamagata S.: Diagnostics of the early gastric cancer. Jap. J. Cancer Clinics, 10: 370-374, 1964.
  - 4) Stuart A.E.: The Cytotoxic Effect of Heterologous Immunized Spleen Cells. III. International Congress of Chemotherapy, 2: 1014-1023, 1963.
  - 5) Soeda M. and Sumiyama, H.: Studies on Carcinogenic Agent of Ehrlich Ascites Tumor. Toho Méd. J., 8: 1600-1609, 1961.
  - 6) Soeda M.: Studies on Immunity against Ehrlich Ascites Tumor. Nat. Def. Med. J. 13: 432-435, 1966.
  - 7) Soeda M.: Studies on Inhibitory Effect of Immunized Spleen Cells on Tumor Growth Nat. Def. Med. J., 13: 539-534, 1966.
  - 8) Soeda M.: Studies on Inhibitory Effect of Homologous Spleen Cells immunized against Ehrlich Ascites Tumor or Yoshida Sarcoma. Nat. Def. Med. J., 13: 550-560, 1966.
  - 9) Soeda M.: Studies on Antitumor Activity of Soedomycin, a New Antitumor Agent. Nat. Def. Med. J., 14: 1-6, 1967.
-