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Immunty in Esophageal Cancer

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Introduction

Many investigators have found depression of immune function in patients with advanced cancer and the immunodeficiency usually increases as the disease progresses. Patients with advanced metastatic disease exhibit profound immunodeficiency, particularly after treatments such as surgery, radiotherapy, or chemotherapy. This immunodeficiency is mainfested by impaired cell-mediated and humoral immunity as well as by impaired nonspecific host defense mechanisms, and it can be measured by both in vitro and in vivo tests[1,2]. Immunodeficiency is also occasionally recognized in the early stages of cancer and correlates with the tumor histology type, the clinical stage, and the response to treatment[3]. These correlations suggest that assessment of the immune response in cancer patients may be helpful in determining mechanisms of tumor development and prognosis. In addition, such studies might aid in detecting early primary or recurrent malignancy, in understanding and augmenting the immune defense mechanisms, and in guiding both conventional therapy and immunotherapy[4]. Immunocompetence is of vital importance when considering the risk of bacterial, viral, and mycotic infection, and also influences tumor dissemination and growth[5,6,7]. Thus, the investigation of immune function is a very important field for cancer therapy.

Esophageal carcinoma shares with pancreatic carcinoma the reputation of being the least curable neoplasms[8]. Although the long-term results and prognosis of esophageal carcinoma have been improved recently through the development of new methods of operation and anesthesia in comparison with gastric or colon cancer, the overall five-year survival rate in esophageal carcinoma is very low (about 20% after esophageal resection). In the Cleveland Clinic experience[9], the 5-year survival rate after curative esophagogastrectomy was 15.4%, with a mean survival time of 34.4 months. There are a number of reasons why the prognosis of esophageal carcinoma is poor. These are the difficulty of diagnosis at an early stage, a high mean patient age, early intraesophageal extension and metastasis, malnutrition due to dysphagia, and the various complications of surgery. Such factors may also impair the immunocompetence of these patients with the tumor itself, malnutrition, age, radiotherapy, chemotherapy and major surgery all contributing to the suppression of host defense mechanisms. It thus becomes possible that the immunocompetence of patients with esophageal carcinoma might be deppressed more early and strongly than that of patients with other gastrointestinal carcinomas.

This review will focus on immunity in esophageal carcinoma.

Skin Tests

Skin tests of recall antigens, nonspecific antigens, or specific antigens (i.e. delayed -hypersensitivity skin reactions) are useful to assess immunocompetence. In one study all control subjects showed a positive dinitrochlorobenzene (DNCB) response, while 20 patients with esophageal carcinoma showed no reaction when they were in negative nitorogen balance. This immunological anergy also persisted when a positive nitrogen balance was achieved[11]. Advani et al. have also reported that the responses to in vivo sensitization with a recall antigen and DNCB were markedly depressed in carcinoma of esophagus being 13% and 16% respectively[12]. We have previously tested the response to four skin tests (Streptokinase -streptodornase (SK-SD), Candida, Phytohemagglutinin (PHA) and Purified protein derivatives (PPD))[13]. The skin test reaction to SK-SD, Candida and PHA were found to be suppressed in patients with esophageal cancer. Furthermore, in the summarized judgment of four types of skin tests in healthy subjects a positive percentage in more than three of the four skin ests was 100%, while in patients of esophageal cancer the positive percentage of that was only 15% (Table 1).

Lymphocyte Blastogenesis

Lymphocyte blastogenesis in response to mitogenic stimulation reflects cell-mediated immunity. In esophageal carcinoma, suppression of the response to PHA stimulation has been reported[8]. We have previously measured PHA-dependent lymphocyte blastogenesis using the whole blood method which we have described previously and found that 78% of the healthy control subjects had a level of more than 10,000 cpm after PHA stimulation, while only 10% of esophageal cancer patients did so[13] (Fig. 1). Thus, cell-mediated immunity was strongly impaired in esophageal cancer.

NK Cell Activity

Lymphocytes which have the capacity to kill syngeneic, allogeneic or xenogeneic tumor cells are designated as natural killer (NK) cells because of their spontaneous response in the absence of any previous sensitization[15]. It has been hypothesized that NK cells may act as surveillance cells by providing a rapid first-line defense against aberrant cells in the body[14,15]. Most of the evidence for the role of NK cells in vitro relates to reduction of the growth of NK -susceptible tumor cell lines. A decrease of circulating NK cell numbers or the impairment of their killer function might have an adverse effect on host resistance to tumors. Talmadge et al. have observed that NK-sensitive B16 tumors grew more slowly and produced fewer metastases in normal mice than in NK-deficient beige mice[15]. Hersy and associates have reported that the incidence tumor recurrence and survival in melanoma patients correlated with NK cell activity[16]. In our study, the NK cell activity of healthy control subjects was 50 ± 2. 89%, while that of advanced esophageal cancer patients was 39.5 ± 7.89%. Furthermore, the NK cell activity of curatively resected cases was significantly higher than

Table 1 Positive percentage of the skin tests in the healthy subjects and the esophageal cancer patients.

Groups \ Skin test	SK-SD*	Candida*	PHA**	PPD
Control	7/14 (50%)	12/14 (86%)	14/14 (100%)	11/14 (78)%
Esophageal cancer patients	4/19 (21%)	6/19 (32%)	9/19 (47%)	6/19 (32%)

*P<0.05, **P<0.01

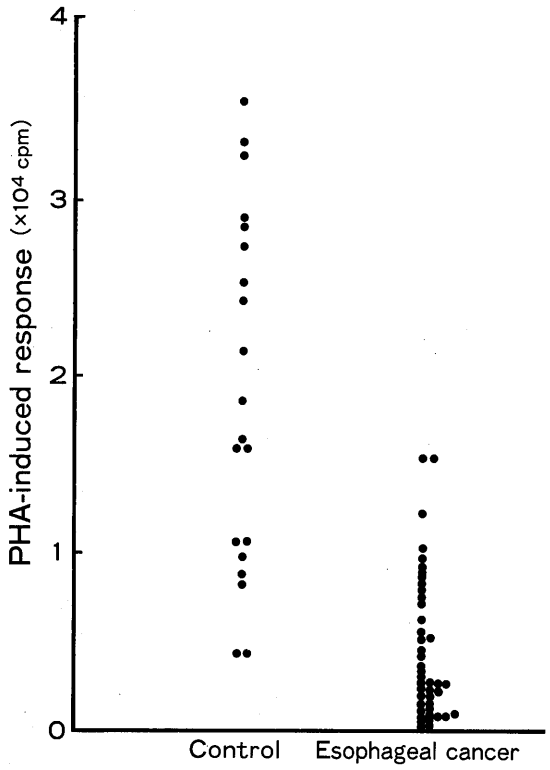


Fig. 1 PHA-induced responses between healthy control subjects and esophageal cancer patients when evalulating lymphocyte reactivity by using the whole blood method. The blood was diluted 16 volumes of RPMI-1640 containing 20% fetal calf serum. Each cultures recieved 30μg/ml PHA. After 96 hour, 5μCi/ml of ^{3}H-thymidine was added and its uptake was measured by a liquid scintillation counter (cpm). The reactivity of lymphocytes from esophageal cancer patients was significantly lower than that of the cells from healthy subjects ($p<0.01$).

of patients undergoing noncurative resection (Fig. 2)[17].

Serum Immunosuppressive Factors

It is well known that there are various immunosuppressive factors detectable in the blood of some individuals with cancer. These factors have been shown to inhibit a number of T cell-dependent immunological

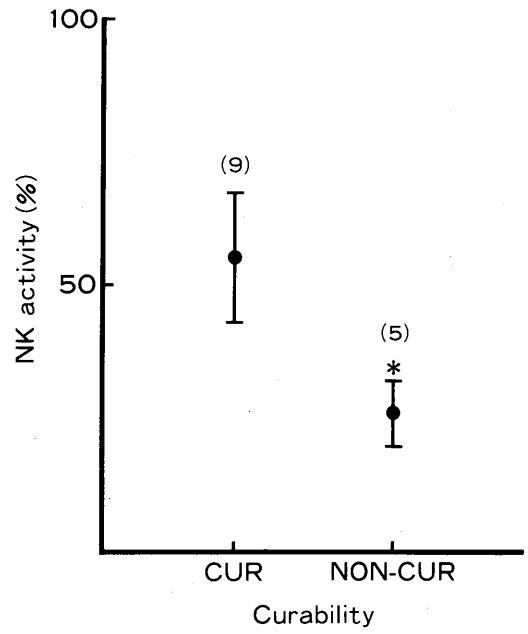


Fig. 2 Comparison of preoperative peripheral blood mononuclear cell NK activity in patients having curative (CUR) or non-curative (NON-CUR) resection of esophageal carcinoma. Values are the group means ± 2 S.E.. *$p<0.01$ vs. CUR.

reaction, including the delayed hypersensitivity response, the rejection of transplantable tumors, and the stimulation of peripheral blood lymphocytes by specific antigens and PHA. Robinson and associates[18] reported that autologous plasma depressed the lymphocyte mitogenic response to PHA in patients with esophageal carcinoma suggesting that this effect of serum might contribute to the immunosuppression of the patients. Tamura has isolated a substance called immuno suppressive acid protein (IAP) from the serum of tumor-bearing mice[19]. We have measured IAP levels in esophageal cancer patints[17]. The IAP level was 375 (363-388) μg/ml in healthy control subjects, and 637 (533-763) μg/ml in patients with advanced esophageal cancer. Moreover, surgical curability was significantly related to IAP levels (Fig. 3)

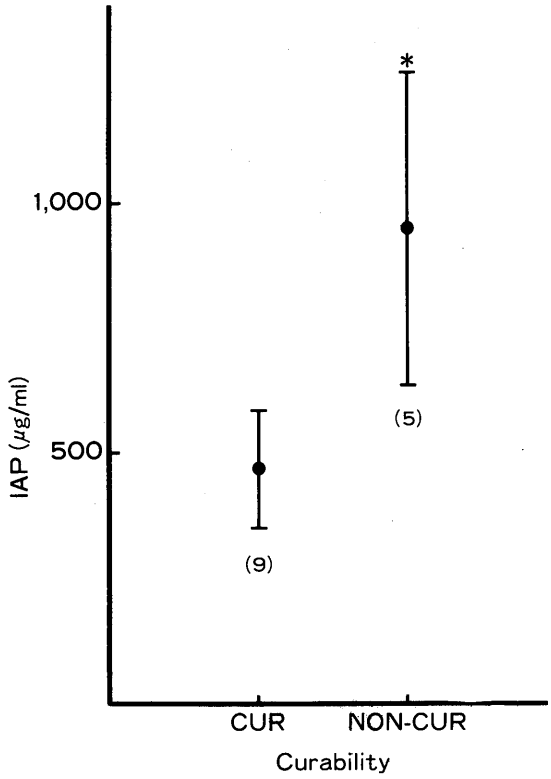


Fig. 3 Comparison of preoperative serum IAP levels in patients with curative (CUR) or non-curative (NON-CUR) resection of esophageal carcinoma. Values are the group means ± 2 S.E.. * $p<0.01$ vs. CUR.

Lymphocyte Subsets

Recently, various lymlphocyte surface markers (including the antigens detected with murine monoclonal antibodies) have been used to categorize lymphocytes with different roles in the immune surveillance system and in the analysis of the lymphocytes that are active in cancer[20.21] and inflammatory diseases. Dillman et al. compared 72 advanced cancer patients and 73 healthy controls, and found that the cancer patients had fewer lymphocytes and helper T cells, but suppressor T cells and Ial cells (which are activated T cells) than the controls[1]. The helper/suppressor cell ratio of the peripheral blood lymphocytes was lower in the cancer group. They selected the combination of the percentage of lymphocyte, the percentage of suppresser cells, the number of helper cells, and the response of pokeweed mitogen stimulation as being the best predictor of immunocompetence in cancer patients. The change in the helper/suppresser cell ratio was thought to be an effect of the tumor. Kasgubowski et al.[21] found a significant decrease in the proportion and number of helper cells in the peripheral blood in patients with solid tumors, as well as an increase in the population of suppressor cells and a decrease in the population of Ial cells in cancer patients. However, our studies found no difference between patients without lymph node metastases and those with metastases in the percentage of CD4 and CD8 cells and the CD4/CD8 ratio[22] (Fig. 4)

Humoral Immunity

Humoral immunity is one of the two major arms of the immunological responses. In cancer patients, serum IgA, IgG, and IgM levels have been examined with conflicting results. Hughes reported that the IgA level was significantly increased in patients with cancer of the mouth, gut and uterus, with IgA/IgG ratio being significantly increased[23]. This finding suggested that there was local rather than systemic antigenic stimulation. In esophageal cancer patients, serum IgA levels were significantly greater than those in control subjects, whereas no significant difference was observed in the levels of IgG and IgM. The complement system has an important role in the immunological defense system. Verhaegen[24] has reported that the complement levels (CH5O, C3, C4, and C1q) of cancer patients were significantly than those of healthy control subjects, and that there was a stage -dependent increase of the complement level. Patients in remission had nearly normal complement levels, patient with localized tumors had raised complement levels, and a further increase was observed in patients with distant metastases. There were no significant differences in the levels of C3, C4, and C3PA between controls and esophageal cancer patients in pretherapeutic period[11.25]. Viral infection has a great influence on im-

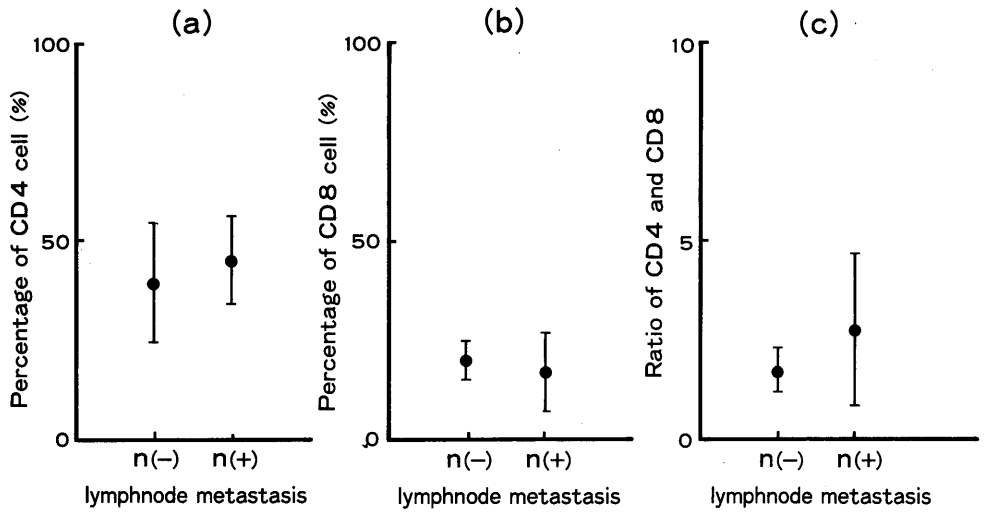


Fig. 4 Comparison of preoperative peripheral blood lymphocyte subpopulations determined using monoclonal antibody in patients with (n(+)) or without (n(−)) lymph nodes metastases of esophageal cancer. No significant difference was observed in the percentage of CD4-positive (a) and CD8-positive (b) cells, or in the CD4/CD8 ratio (c). Values are the group means± 2 S.E..

munocompetence and there are some reports which indicate a possible relationship between esophageal carcinoma and viral infection[26.27]. Therefore, the humoral immunity is one of the important factors to assess the immune status.

Nutrition

Narrowing of the esophageal lumen by a tumor can cause difficulty in the swallowing of solides, soft food, pursed food, or even liquids. The resultant decrease in oral intake contributes to specific nutritional deficits and generalized weight loss. In carcinoma of the esophagus, the immune response can thus be depressed by two main factors, i.e., the presence of the malignant tumor itself and malnutrition. McFarlane and Hamid reported many years ago that lymphocyte ability to form rosette to sheep red blood cells and lymphocyte blastogenesis were impaired in rats with malnutrition[28]. We observed the immunocompetence in rats with esophageal cancer induced with N-butyl -N-nitrosourethan. All rats suffered from malnutrition due to dysphagia caused by esophageal cancer or papilloma. PHA-induced lymphocyte blastogenesis of the peripheral blood lymphocytes in rats decreased significantly[28]. Chandra has reported that the frequency of circulating rossette-forming thymus-dependent lymphocytes was reduced in malnourished infants and children[30]. It has been reported that reversal of the nitrogen balance resulted in a significant increase in T-lymphocytes overall in T cell numbers, as well as a significant increase in the mitogenic response to PHA[11]. Daly et al. demonstrated a significant reduction in major wound infections, other infection, and general postoperative complications in esophageal cancer patients who received preoperative total parental nutrition (TPN) for at least 5 days in comparison with the postoperative TPN and non-TPN groups (4% vs, 24% and 23%, respectively)[31].

Influence of Surgical Stress

In general, surgical stress is reported to impair host defenses. Immunocompetence is of vital importance in a surgical patient, as it influences on the individual's susceptibility to

becterial, viral, and mycotic infections[5,6]. In cancer patients immunocompetence can also influence on tumor dissemination after surgry[7]. Usually, thoracotomy and laparotomy are performed during operations on esophageal carcinoma. Hattori et al. have reported that thoractomy and laparothoracotomy significantly reduced the survival time of inoculated rats in comparison with control animals[32]. We studied the behavior of peripheral mononuclear cells (PBMC) in 12 laparotomy patients and 6 thoracotomy

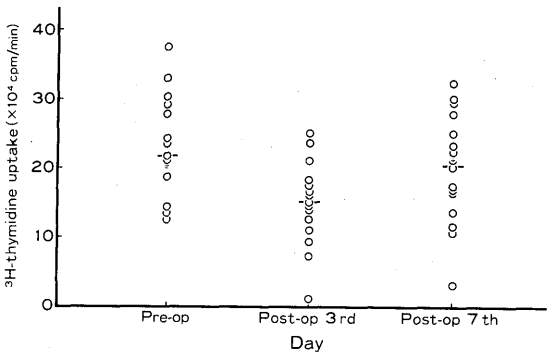


Fig. 5 Postoperative change in PHA blastogenesis. ^{3}H-thymidine uptake was used for measuring PHA blastogenesis. Pre-op vs. Post-op 3rd day, $p<0.002$. Pre-op vs. Post-op 7th day, NS. Post-op 3rd vs. Post-op 7th day, $p<0.006$

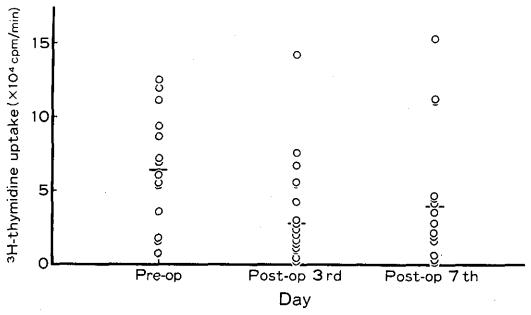
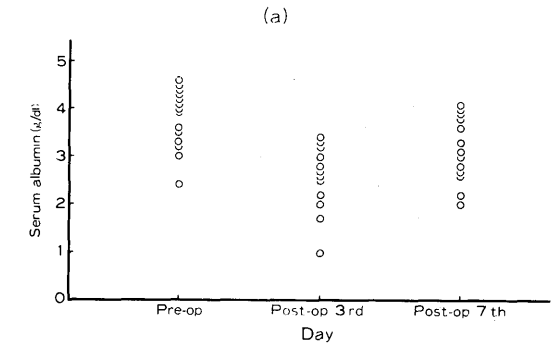
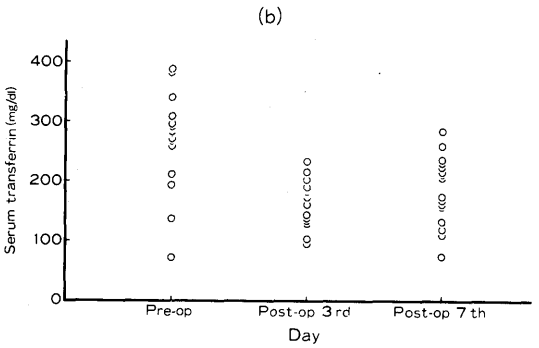


Fig. 6 Postoperative change in SAC blastogenesis which was determined by ^{3}H-thymidine uptake. Pre-op vs. Post-op 3rd day, $p<0.0002$. Pre-op vs. Post-op 7th day, $p<0.02$. Post-op 3rd day vs. Post-op 7th day, NS.





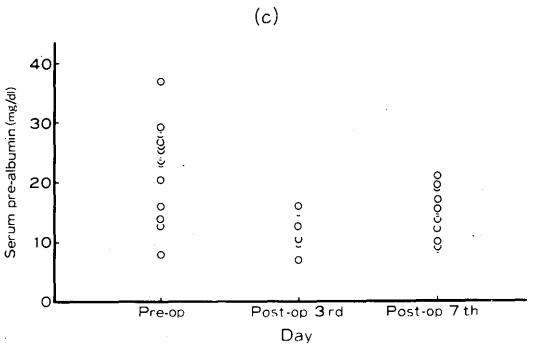


Fig. 7 (a) Postoperative change in serum albumin. Pre-op vs. Post-op 3rd day, $p< 1 \times 10^{-6}$. Pre-op vs. Post-op 7th day, $p<0.0004$. Post-op 3rd day vs. Post-op 7th day, $p<0.009$. (b) Postoperative change in serum transferrin. Pre-op 3 rd day, $p<4 \times 10^{-6}$ 7th day, $p<0.05$. (c) Postoperative change in serum pre-albumin. Pre-op vs. Post-op 3rd day, $p<1 \times 10^{-6}$. Post-op 7th day, $p<1 \times 10^{-6}$. Post-op 3rd vs. Post-op 7th day, $p<0.005$.

patients[33]. Immunocompetence was evaluated by the blastogenesis of PBMC in response to PHA and staphylococus Cowan I (SAC). Nutritional parameters (albumin, transferrin, and prealbumin levels) were evaluated preoperatively, on the 3rd and 7th postoperative days. PHA blastogenesis was depressed significantly on the 3rd postoperative day (Fig. 5). The response to SAC and the nutritional parameters were both decreased significantly on the 3rd and 7th postoperative days (Fig. 6 and 7). These results make it clear that surgical sterss impairs both T and B cell function, as well as nutrition.

Summary

Patients with esophageal carcinoma have a definite decrease in immunocompetence in the preoperative period and these patients may undergo major surgery, radiotherapy, or chemotherapy. Therefore, esophageal carcinoma patients may develop profound immunosuppression after treatment. For the prevention of immunosuppression, specific immunotherapy and nutritional support should be considered.

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