Cytokine response following transthoracic and transhiatal esophagectomy in patients with esophageal cancer

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ABSTRACT. Curative esophageal resection is usually performed using either a transthoracic (TT) or transhiatal (TH) approach. Forty patients with esophageal squamous cell carcinoma who underwent esophagectomies (24 TT and 16 TH), 12 patients who underwent surgery for gastric cancer, and 16 healthy individuals were enrolled in this study. Blood samples were taken from the patients, pre- and post-surgery. The levels of synthesis of T-helper 1 and 2 cytokines were assessed *in vitro* in the presence of mitogen. Our initial data indicated that at admission, 24 h before surgery, blood cells from both groups of esophageal cancer patients produced significantly lower levels of the Th1 cytokines, IFN-γ and IL-2 than those from cells of healthy donors. Cells collected from gastric cancer patients prior to surgery produced intermediate levels of IFN-γ and IL-2: significantly lower than healthy donors, and slightly more (non-significant) than esophageal cancer patients. These results contrast with those for the production of Th2 cytokines prior to surgery, which did not differ significantly between any groups: either the esophageal or gastric cancer patients, or healthy donors. Th1 and Th2 cytokine production was then studied using blood cells collected seven days after surgery. Cells from both groups of esophageal cancer patients, undergoing either TT or TH surgery, produced significantly lower levels of the Th1 cytokines, IFN-γ and IL-2 than those from cells of gastric cancer patients who had undergone surgery. Postoperative and preoperative production was compared. For patients who had undergone TT esophageal resection, we observed that the post-operative production of IL-2, IL-5 and IL-13 was significantly lower than the pre-operative production of those cytokines. Such reduced post-operative compared to pre-operative production was only significant in patients who had undergone TT esophagectomy. A similar, but non-significant trend was observed in patients who had undergone either TH esophagectomy, or gastrectomy. The results indicate that digestive tract cancer patients, both esophageal and gastric, have (prior to surgery), a significantly reduced, basal, mitogen-induced production of Th1 but not of Th2 cytokine. Post-operatively, a significantly reduced production of Th1 and Th2 cytokines, except for IFN-γ, was observed only in patients who had undergone surgical esophageal resection using the TT method.

Keywords: cytokine, interferon gamma, interleukin-2, esophagectomy

Esophageal cancer is a serious malignancy of the digestive tract with a fatal outcome in a majority of cases [1]. Surgical treatment, plus chemotherapy, is considered to provide one of the best chances for cure in these patients. Curative esophageal resection may be performed using a transthoracic (TT) or transhiatal (TH) approach [2]. The first strategy is aimed at optimising the cure rate through extensive mediastinal and abdominal lymph node dissection. The other strategy, where the esophagus is resected using a cervico-abdominal approach, without formal thoracotomy, is intended to decrease early postoperative risk of morbidity and mortality [2, 3]. There has been much controversy about the appropriateness and long-term survival rates with TT and TH resection in esophageal cancer

patients [4-6]. In fact, it is unclear which approach has the greater benefit in terms of patient survival. As regards postoperative mortality or morbidity rates, a few studies indicate that the outcomes following the TT and TH approach are equivalent [7, 8].

After surgical trauma, an appropriate T helper (Th) cell response is critically important in the immune reaction [9, 10]. Th lymphocytes are functionally subdivided into Th1 and Th2 cells. Th1 cell response is further characterized by the production of cytokines such as IFN-γ, IL-2 and TNF-β (lymphotoxin), and Th2 cell response is primarily characterized by secretion of IL-4, IL-5, IL-10 and IL-13 [11, 12]. In experimental studies, both Th1 and Th2 cells have a role in orchestrating the host immune response to

tumor [13]. IFN-γ and IL-2 are involved in host defense against bacteria, viruses and fungi; these cytokines activate monocyte/macrophages, natural killer cells and cytotoxic T lymphocytes [12, 14]. Experimental and clinical studies indicate that surgical or traumatic injury alter immune reactions including monocyte functions and the ability of T-lymphocytes to proliferate in response to mitogenic activation, i.e. concanavalin A (Con-A) or PHA [15-18]. These studies have shown the postoperative depression of the mitogenic response of lymphocytes in patients and animals, and that the extent of the immunosuppressive effects is correlated with the complexity of the surgery [16-18]. The purpose of the present study was to investigate the differences between TT and TH esophagectomies with respect to Th1 and Th2 cytokine production. We conducted a prospective study involving 40 patients who underwent elective esophageal surgery (transthoracic or transhiatal). The levels of cytokines measured for these groups were compared with those of a group of patients undergoing gastrectomy and a group of healthy individuals.

PATIENTS AND METHODS

Subjects

Between March 2006 and June 2007, 74 consecutive, esophageal squamous cell carcinoma (ESCC) patients were admitted for elective surgery to the Division of Surgery, Cancer Institute, Medical Sciences/University of Tehran. After excluding those patients who did not match the criteria for this study, 40 ESCC patients who subsequently underwent esophagectomies were prospectively recruited into the study. These patients were assigned to undergo esophagectomy using either a transthoracic technique (group 1; 24 patients) or a transhiatal approach (group 2; 16 patients). Meanwhile, in order to rule out the effects of surgical injury and the malignancy, we selected a group of patients with gastric cancer undergoing surgery; this group of 12 patients, undergoing surgical gastrectomy with lymph node dissection, was prospectively entered into the study (group 3). Moreover 16, age- and sex-matched, healthy individuals were also enrolled this study (group 4). Written informed consent was obtained from each patient before surgery and the study was approved by the Hospital's Ethics Committee. The eligible cases had histologically confirmed, squamous cell carcinoma of the mid-to-distal esophagus, and had no evidence of distant metastases. Exclusion criteria for all patients, including control groups, were previous gastric or esophageal surgery, previous chronic organ insufficiency as defined by ASA III or IV (American Society of Anaesthesiology) [19], neoadjuvant radio- or chemotherapy, and if it were not possible to construct a gastric tube. Patients with immune or central nervous system dysfunction or patients with congestive heart failure, malnutrition, diabetes, infection, or inflammation were also excluded. Similarly, patients who developed postoperative complications were not included in the study. All patients received general anesthesia during surgery using a standardized, analgesic regime. Tumors were removed using either a TT or TH approach, which was decided by the attending surgeon on an individual basis. Postoperatively, patients were placed under intensive care unit surveillance for 24 to 48 h.

Blood sampling

Venous peripheral blood samples were obtained from all subjects, and blood was collected in polystyrene, round-bottomed, sterile tubes with caps, containing lithium-heparin (15 IU Li-heparin/ml blood, Leo Pharmaceutical Products, Eeesp, The Netherlands). Blood samples were taken 24 hours before surgery and seven days after. Blood sampling was performed for all patients between 8 and 10 a.m., and blood cultures were started within 1 h of collection.

Whole blood cell culture

Whole blood was diluted 1:10 in culture medium consisting of RPMI-1640 (Gibco Life Technologies, Paisly, UK), 2 mM L-glutamine, penicillin (100 IU/mL) and streptomycin (100 μg/mL). The diluted blood was placed in 24-well culture plates, 1 ml/well. The cells of diluted whole blood were either cultured alone as control or stimulated with Phytohemagglutinin (PHA, 5 μg/mL) (L9017, Sigma, Sweden). Mitogen was used at the optimal stimulatory concentration as described elsewhere [20]. Both mitogen-stimulated and control cultures were run in duplicate wells. The plates were incubated in a humidified air atmosphere at 37°C with 5% CO_2 for 72 hours. After incubation, supernatants were removed, centrifuged at $300 \times g$ and kept frozen at -20°C until assayed for cytokine concentrations.

Cytokine measurements

The frozen culture supernatant fluids were thawed at room temperature and cytokines, IFN-γ, IL-2, IL-5, IL-13 levels were measured by enzyme-linked immunosorbant assay (ELISA) using commercial assay kits supplied by U-CyTech Biosciences (The Netherlands), according to the manufacturer's instructions. The assays were performed identically except that different coating and detector antibodies were used. The absorbance of each well was read at 450 nm and cytokine concentrations in the samples were calculated with a standard curve generated from recombinant cytokines. Cytokine values were expressed as picograms/milliliter (pg/mL). Cytokine levels in the supernatants of all stimulated cells from diluted whole blood were above the lower detection limit of the measured cytokines (> 8 pg/mL). While there was no measurable levels (> 1 pg/mL) of any of the determined cytokines in the supernatants of unstimulated cultures, the results of this study are presented based on stimulated cells.

Statistical analysis

Statistical analysis of data was performed using SPSS, version 15. The results were presented as the mean ± standard deviation. Comparison of means before and after surgery was performed by the paired-samples t-test. The one-way ANOVA test was performed to compare the means among the groups. P-values of less than 0.05 were considered statistically significant.

RESULTS

Patient characteristics

Detailed information about the patient groups who underwent esophagectomy either using the transthoracic surgical approach or the transhiatal procedure, and the group of patients undergoing gastric surgery is provided in *table 1*. The patient groups were comparable in age, sex and preoperative tumor staging *(table 1)*. Although the mean operating time in group 1 was significantly higher than group 2 (357 *versus* 223 minutes, p < 0.01), there was no difference in mean hospital stay between the two groups. There was no 30-day mortality in the patients studied.

Preoperative cytokine production

Data for Th1- and Th2-type cytokines in the patient groups are presented in *table 2*. There was no significant difference between preoperative levels of any of the cytokines measured (IFN-γ, IL-2, IL-5, and IL-13) among the patient groups. However, Th1 cytokine levels in the control group (group 4) were significantly higher than in the patient groups (143.56 ± 35.92 *versus* 54.58 ± 33.93 pg/mL for IFN-γ, p < 0.001; 241.63 ± 27.68 *versus* 160.42 ± 21.08 pg/mL for IL-2, p < 0.001), but there was no significant difference in Th2 cytokine levels *(figure 1)*. Although the preoperative levels of Th1 cytokines in group 3 were higher than in groups 1 and 2, these differences were not

significant (p = 0.258 for IFN-γ and p = 0.134 for IL-2) *(figure 2)*. Moreover, there was no difference between Th2 cytokine levels among these groups (p = 0.696 for IL-5 and P = 0.304 for IL-13) *(figure 3)*.

Postoperative cytokine production

There was significantly lower, postoperative production of IFN-γ and IL-2 in esophagectomy patients (groups 1 and 2), compared with patients who had undergone gastrectomy *(table 2, figure 2)*. Although the production of IL-5 in mitogen-induced, culture supernatants in esophagectomy patients (groups 1 and 2) was lower than in the gastrectomy subjects (group 3), the difference was not significant *(table 2, figure 3)*.

Pre- and post-surgical comparison of cytokine production

The Th1 cytokine (IFN-γ) levels in mitogen-induced culture supernatants were very simiar to preoperative levels in group 2, but this value decreased in group 1 *(table 2, figure 2)*. IL-2 levels were reduced significantly in patients who underwent the transthoracic procedure (group 1) compared with preoperative levels; however, the reduction in IL-2 levels compared with preoperative levels in group 2 was not significant *(table 2)*. Levels of IL-5 in patients from both groups 1 and 2 were significantly lower than the preoperative levels, whereas in group 3, the reduction in

Table 1
Characteristics of patients who underwent esophageal resection using a transthoracic
or a transhiatal approach, and patients undergoing gastrectomy

Characteristics	Transthoracic (n = 24)	Transhiatal (n = 16)	Surgical gastrectomy (n = 12)
Age (years)			
Mean	58	65	62
Range	38-82	43-82	45-70
Sex (male/female)	13/11	9/7	7/5
Hospital stay (days)*			
Mean	20	19	15
Range	6-34	8-29	10-29
Duration of surgery (minutes)			
Mean	357	223	172
Range	300-455	180-280	120-240
Duration of Anesthesia (minutes)			
Mean	427	279	214
Range	360-515	225-330	160-280

* "Hospital stay" was defined as the number of days from the day of surgery to discharge.

Table 2
In vitro cytokine levels (pg/mL) of PHA-induced whole blood cells from patients who underwent esophageal resection using either a transthoracic or transhiatal approach, and patients undergoing gastrectomy, prior to surgery and on the 7th postoperative day

Cytokines	Transthoracic (n = 24)			Transhiatal (n = 16)			Gastrectomy (n = 12)			P-value**
	Before	After	p*	Before	After	p*	Before	After	p*	
IFN-γ	54.29 ± 48.33	38.25 ± 23.80	0.147	45.63 ± 10.04	46.19 ± 13.21	0.871	67.08 ± 5.14	75.00 ± 12.43	0.014	< 0.001
IL-2	160.29 ± 17.26	145.71 ± 20.23	0.002	153.62 ± 22.74	146.06 ± 22.87	0.162	169.75 ± 23.86	166.75 ± 28.69	0.477	0.030
IL-5	22.58 ± 6.88	19.63 ± 4.58	0.002	21.31 ± 5.15	17.81 ± 2.32	0.009	23.33 ± 6.99	21.08 ± 5.43	0.166	0.135
IL-13	45.92 ± 12.13	37.38 ± 9.53	0.012	46.56 ± 15.14	39.31 ± 9.17	0.124	39.33 ± 13.24	34.42 ± 10.82	0.240	0.425

* P-value between before and after surgical resection.
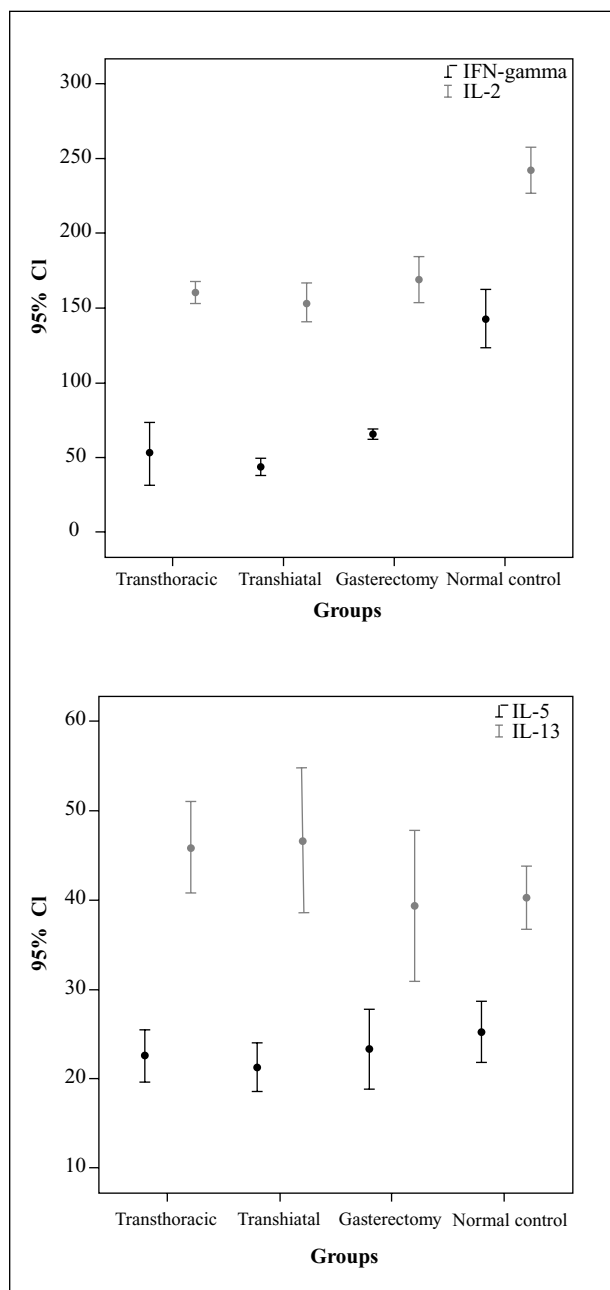** P-value among groups after surgical resection.

Figure 1
Error bar for Th1 and Th2 cytokine production (pg/mL) in the four groups prior to surgery.

Figure 2
Error bar for Th1 cytokine production (pg/mL) in patients who underwent transthoracic or transhiatal esophageal resection, and patients undergoing gastrectomy, pre- and post-surgery.

IL-5 levels compared with the preoperative levels was not significant *(table 2)*. There was a significant decrease in the levels of IL-13 in group 1 compared with preoperative levels, while in group 2, the reduction in IL-13 compared with preoperative levels was not significant *(table 2)*.

DISCUSSION

There are a number of studies on lymphocyte subsets or Th1/Th2 immune responses in patients with esophageal carcinoma with conflicting results [21-24]. Most of these studies were concerned with preoperative levels in order to be able to define surgical resectability, and predict clinical outcome and immune function [21, 24]. Few reports have documented postoperative Th1/Th2 immune function
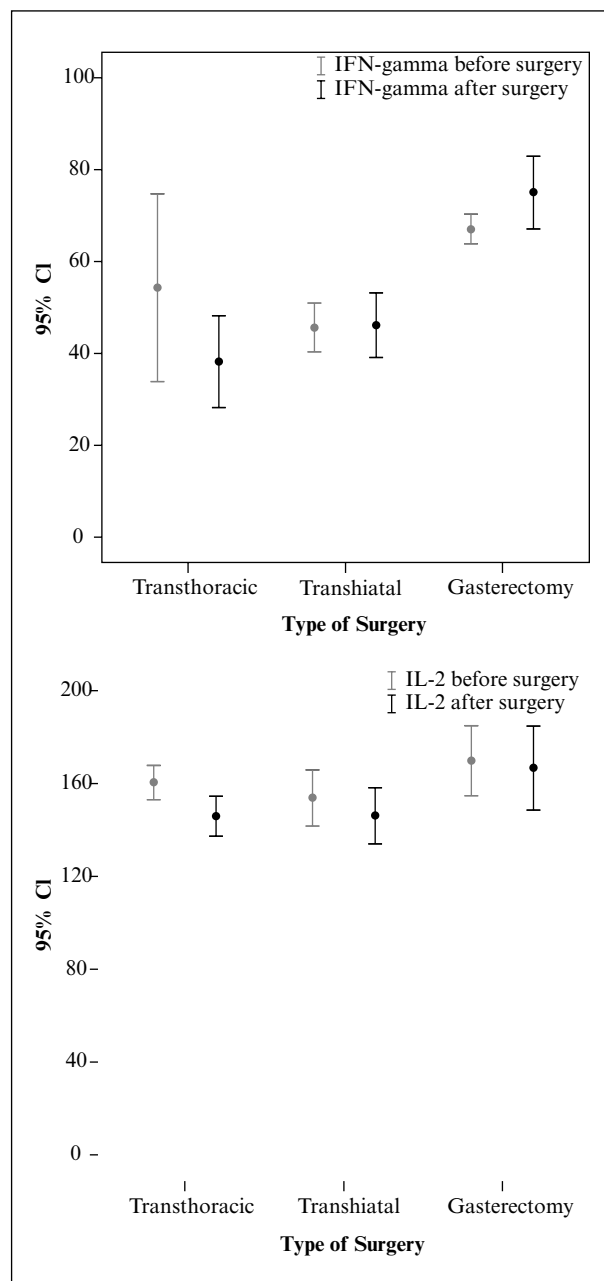
[22, 23]. The current study was performed to see if there were any differences between the two major approaches for esophageal resection in cancer patients, with regard to Th1- and Th2-type cytokine production by induced lymphocytes. The results clearly demonstrate that surgical esophageal resection, by either of the two approaches, causes suppression of CD4+ T helper cell proliferation and secretion of both Th1- and Th2-type cytokines, including IFN-γ, IL-2, IL-5 and IL-13.

Contradictory reports exist on the cytokine response of Th1/Th2 cells in patients following major surgery. A report from Berguer and coworkers indicates a postoperative reduction in Th1 cytokine secretion without a shift in the balance toward Th2 cytokine predominance [18]. Some other authors have noted a deficiency of the Th1 cytokine
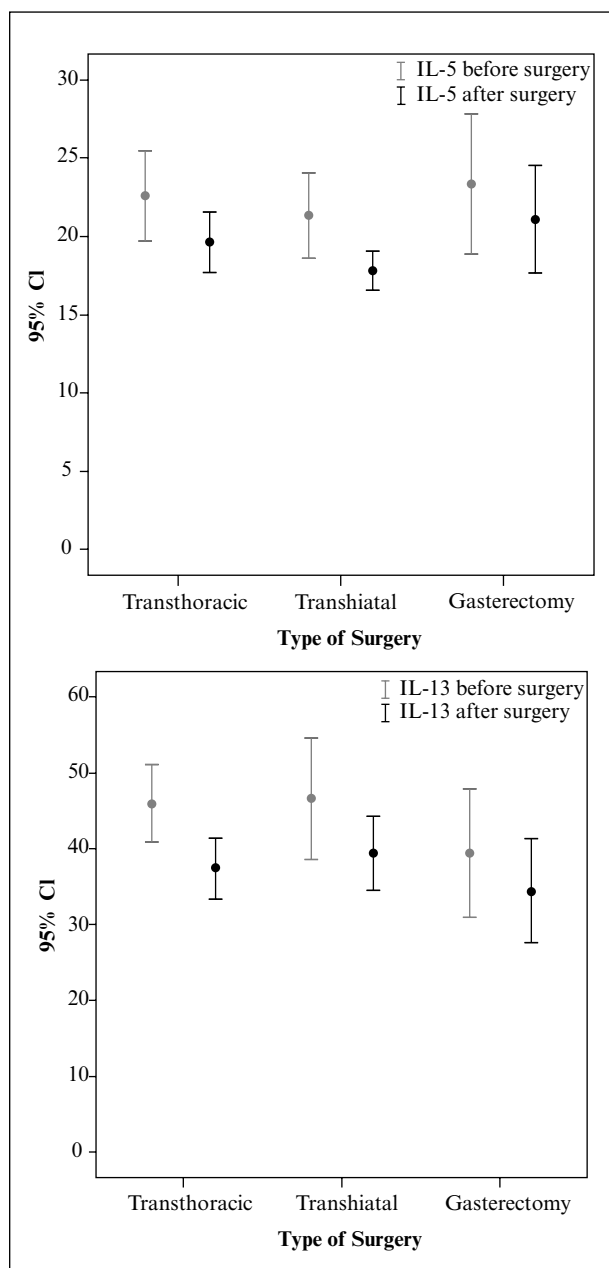
Figure 3
Error bar for Th2 cytokine production (pg/mL) in patients who underwent transthoracic or transhiatal esophageal resection and patients undergoing gastrectomy, pre- and post- surgery.

response, associated with increased production of Th2 cytokines, in patients exposed to surgical trauma [25]. Similar, postoperative immune function alterations have been reported in a series of patients, including esophageal cancer cases, by Hensler *et al.* who demonstrated a very marked defect in the T-cell cytokine production response, for both Th1 and Th2 cells, during the postoperative period [17]. Also, the current results are consistent with the results of Koenig and coworkers, who showed that cell-mediated immunity (investigated by *in vitro* mitogen/antigen-induced lymphocyte proliferation) was depressed for 3-10 days postoperatively in healthy patients (without trauma) who underwent major surgery, but not following minor surgery [26]. In this study, the esophagectomy patients in both groups had lowered secretion of induced IFN-γ and IL-2. These cytokines are involved in the devel-

opment of the Th1 subset of lymphocytes, and are responsible for cell-mediated immunity. Likewise, as described by Faist *et al.*, immunosuppression after major trauma results mainly from T-cell dysfunction and is characterized by impaired synthesis of IL-2 and IFN-γ [27]. The postoperative reduction in Th1-type cytokine secretion was also demonstrated by van Sandick and colleagues who found that the two major surgical procedures, TT and TH, had a similar suppressive effect on Th1-type responses. In addition, they found that Th2-type responses were less affected by the TH procedure than by the TT technique [22]. The findings of our study also indicated that post-operative IFN-γ production in those patients who underwent TH resection, was similar to the preoperative rate, and that the reduction in IL-2 secretion was not significant in this group. However, we observed a significant reduction in IL-2 in the TT esophagectomy group and a decline in IFN-γ production. Thus, the TH esophagectomy procedure seemed to have a less suppressive effect on the Th1 cell response than the TT approach.

The results of the current study are consistent with those of Ogawa *et al.*, who found that in patients exposed to surgical stress, peripheral blood lymphocyte numbers and function were suppressed until at least two weeks postoperatively, and that this immunosuppression was mainly due to a decrease in helper/inducer T cells, cytotoxic T cells, interlukin-2 receptor-positive cells, and an increase in suppressor T cells [25]. These investigators concluded that, with the decrease in the lymphocyte count and the changes in peripheral blood lymphocyte subsets, immunocompetent cell function decreases and cellular immunity is suppressed [25].

The data in the present study show that the duration of anesthesia and the length of surgery are significantly longer in the TT approach than the TH approach. While the duration of anesthesia in these two approaches could affect cytokine production, some previous studies that investigated the effect of the general anesthesia on the *in vitro* production of proinflammatory cytokines [28, 29], indicated that anesthesia promotes production of proinflammatory cytokins such as IFN-γ, TNF-α and IL-1 β, enhances the percentage of T and B cells, and that opioid-based anesthesia can increase the gene expression of proinflammatory cytokines in alveolar macrophages.

Our findings indicate that the PHA-induced cytokine production in esophagectomy patients decreased by the 7th postoperative day compared with patients who had undergone gastrectomy. Similarly, Tashiro and colleagues, who investigated the immune function of patients following surgery for esophageal carcinoma in comparison with immune function following gastric surgery, found that in the patient group undergoing esophagectomy, PHA-stimulated lymphocyte proliferation had decreased significantly by the 7th postoperative day, and remained at low levels until three weeks after surgery. However, in the patient group that underwent gastric surgery, lymphocyte proliferation decreased but not significantly by the 7th postoperative day, and then returned to normal by three weeks post-surgery [30]. These authors suggest that several factors may account for the decreased lymphocyte proliferation in esophagectomy patients, such as the duration of surgery and anesthesia, the extent of tissue injury and blood loss, which are obviously far greater in patients undergoing esophagectomy [30].

The data in this study indicate that the preoperative levels of induced cytokines, apart from IL-13, are lower than those in healthy individuals. This state is presumably indicative of a severe, host immune suppression in esophageal cancer patients. Published data on the immune status prior to surgery are also conflicting. A published report indicates that prior to surgery, patients with adenocarcinoma of the esophagus or esophagogastric junction have an up-regulated Th1 response compared with healthy volunteers [21]. Another report suggests that a Th2-dominant immune state may occur in cancer patients, based on the findings that the proportions of CD4$^+$ T-cells that produce cytokines such as IL-4, IL-6, and IL-10, are significantly higher in peripheral blood from patients with digestive cancer than that from healthy controls, but without a decreased secretion of Th1-type cytokines such as IFN-γ [31]. An additional study has indicated the suppression of immune function in cancer patients [32]. These observations in cancer patients may be due to the heterogeneity of the patient groups and the extent or the stage of the disease. The differences in mortality or morbidity, and postoperative complications between these two approaches for esophagectomy, are contradictory. Hulscher and coworkers randomly assigned 220 patients with adenocarcinoma of the esophagus or adenocarcinoma of the gastric cardia involving the distal esophagus, either to TT esophagectomy with extended en bloc lymphadenectomy or to the TH approach. They concluded that TH esophagectomy was associated with lower morbidity than the TT approach. However, overall disease-free, and quality-adjusted survival did not differ statistically between these two groups of patients [33]. Orringer *et al.* retrospectively reviewed 1 085 patients with intrathoracic esophageal disease, and concluded that TH resection has less morbidity than TT resection [34]. Some other authors found no significant differences in postoperative mortality or morbidity between TT and TH esophagectomies [7, 8, 35].

In conclusion, the results of the present study indicate suppression of T-cell function following surgical resection of the esophagus in cancer patients, and in terms of induced cytokine production by Th1/Th2 cells, of the two surgical approaches for esophagectomy, the transthoracic procedure is associated with a more severe reduction in Th1-type cytokines following surgery, than the transhiatal approach.

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