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Clinical significance of autologous tumor killing (ATK) activity and its induction therapy in human cancer

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Abstract

The activity of blood lymphocytes to kill autologous freshly isolated tumor cells tested at the time of surgery predicts a favorable clinical course in patients who have primary localized solid tumor and receive curative operation. The strong correlation of autologous tumor killing (ATK) activity with disease-free interval and total survival indicates that ATK activity is a meaningful prognostic indicator and provides evidence for immunological control of tumor growth and metastasis. Although there is no direct evidence that ATK lymphocytes play a critical role in regression of tumor and prevention of tumor regrowth, the lack of ATK activity in patients who relapsed and died may not result from other factors related to their poor performance status, immune functions and tumor characteristics. Clinical trials with ATK induction therapy resulted in an improvement of the clinical outcome in patients who naturally have no such potential. The data indicate that the presence of both natural and induced ATK activity is strongly associated with long-term survival. In addition, adoptive transfer of BRM-induced ATK effector cells resulted in prolongation of survival time even in patients with documented metastatic tumors. Thus, considerable emphasis should be placed on a strategy that induces ATK activity *in vivo*. Such an approach may provide a new focus for cancer immunotherapy.

Abbreviations: ATK: Autologous tumor killing; BRM: biological response modifiers; AIDS: acquired immune deficiency syndrome; NK: natural killer; LGL: large granular lymphocytes; TIL: tumor-infiltrating lymphocytes; MHC: major histocompatibility complex; TCR: T cell antigen receptor; LAK: lymphokine-activated killer; IL: interleukin; IFN; interferon; TNF: tumor necrosis factor; ATKF: autologous tumor killing factor; LFA-1: leukocyte function-associated antigen 1; ICAM-1: intercellular adhesion molecule 1; mAb: monoclonal antibodies

Introduction

The host immune defense system is thought to prevent a single malignant cell from developing into tumors. If such systems did not exist, the incidence of tumor occurrence would be much higher. In fact, the increased incidence of malignant tumors has been observed with patients receiving continuous immunosuppressive treatment following organ transplantation, and the high frequency of Kaposi's sarcoma other types of malignancies was documented in patients with AIDS (acquired immune deficiency syndrome). Such evidence was often cited in support of the concept that immune surveillance mechanisms operate against human malignant diseases.

A variety of components of adaptive and natural immunity, including T cells, natural killer (NK) cells, K cells, monocyte/macrophages, granulocytes, and mast cells may play a role in immune resistance against tumor [1, 2], which leads the following predictions (Table 1):

1) Tumor cells express antigens or structures that are recognized by one or more effector cell types. In fact, the same tumor cell was recognized by autologous T cells, NK cells and monocyte/macrophages [3–5].

Table 1. Actual version of immune surveillance.

1.	Tumor cells express antigens or structures that are recognized by one or more effector cells.
2.	Tumor cells are sensitive to cytotoxicity or growth inhibition by one or more effector types.
3.	One or more relevant effector cells infiltrate the site of tumor growth.
4.	Activation of relevant effector cell types may reduce the incidence of tumor or metastasis and prolong survival time.
5.	Suppression of relevant effector mechanisms may elevate the incidence of tumor and accelerate tumor development.

2) Tumor cells are sensitive to cytotoxicity or growth inhibition mediated by one or more effector cell types. Indeed, fresh human tumor cells are killed by autologous T cells [6–10], large granular lymphocytes (LGL) [11, 12], activated killer cells [13–15], and soluble cytotoxic factors [16, 17].
3) One or more of the relevant effector cells infiltrate the site of tumor growth. In fact, a variety of effector cells have been observed within or around the tumor site of cancer patients, and tumor-infiltrating lymphocytes (TIL) and monocytes exhibit cytotoxicity against autologous tumor cells *in vitro* [8, 18, 19, 20].
4) Activation of relevant effector cell types may reduce the incidence of tumor or metastasis, prolong survival time and lead to an eventual rejection of tumor [4, 21, 22].
5) Suppression of relevant effector mechanisms may elevate the incidence of tumor and accelerate the development of tumor. Taken together, the actual version of the immune surveillance theory involves the assumption that malignant cells express antigens or structures that are recognized by one or more immune effector cell types and that these various effectors could eliminate tumor cells.

In vitro cytotoxicity is considered to be one expression of host immune defense against tumor. The outcome of cytotoxicity assays depends on a variety of factors, including the immunological history of cancer patients, previous exposure to agents, physical and psychological stress of patients, the activation profile and composition of lymphocytes, and the characteristics of tumor cells. All of these factors may represent a balance of the cytolytic potential of various effector cells and innate susceptibility of tumor cells. Because of the complexity of the immune system, however, the results obtained *in vitro* cytotoxicity tests do not necessarily reflect *in vivo* events. Accordingly, the failure of *in vitro* demonstration of tumor cell lysis does not necessarily rule out the existence of an anti-tumor immune response in the cancer patient. Conversely, the positive results in *in vitro* cytotoxicity tests does not necessarily imply that immune destruction of tumor cells occurs *in vivo* in the patients.

Most studies on cell-mediated cytotoxicity against tumor have been performed by the use of tumor cell lines as targets. It is, however, difficult to interpret the data on cytotoxicity against cultured tumor cells, since *in vitro* culture of tumor cells may alter their susceptibility to cell-mediated lysis through several mechanisms (Table 2) [2, 22]:

1) Tumor cells that are adapted to *in vitro* culture conditions may selectively grow.
2) Tumor cells may lose putative tumor-associated and/or major histocompatibility complex (MHC) antigens that have previously been expressed on the tumor cells *in vivo*.
3) Tumor cells may acquire a new antigen(s) and/or structure(s) that does not exist *in vivo*. Few researchers, however, have used freshly isolated human tumor cells as targets in cytotoxicity assays because of the difficulty to obtain fresh tumor cells with high purity and good viability (Table 2).

1) Enzyme treatment of tumor samples that are required for isolation of tumor cells may alter the surface characteristics.
2) Mechanical treatment may cause damage of tumor cells.
3) Some of the contaminating stromal cells instead of tumor cells may serve as targets.
4) Contamination with lymphoid cells and monocyte/macrophages may function as effectors and influence cytotoxicity. In addition, the autologous combination of effector and target cells is of critical importance in the cytotoxicity assay since T lymphocytes recognize tumor cells through interaction of T cell antigen receptor (TCR)/CD3 complex and MHC/tumor antigens [6, 8, 9]. For better understanding of the cytotoxic function of lymphocytes against tumor, it is desirable to perform a cytotox-

Table 2. Advantages and difficulty of use of fresh human tumor cells in cytotoxicity tests.

Advantage	
1.	To void selective growth of tumor cells that are adapted to *in vitro* culture conditions.
2.	To avoid loss of antigens that are expressed *in vivo* on tumor cells.
3.	To avoid acquisition of new antigens that do not exist *in vivo* in tumor cells.
Difficulty	
1.	Enzyme treatment of tumor samples that are required for isolation of tumor cells may alter the surface characteristics.
2.	Mechanical treatment may cause tumor cell damage.
3.	Contaminating stromal cells instead of tumor cells may serve as targets.
4.	Contamination with lymphoid cells and monocyte/macrophages may influence cytotoxicity.

icity assay with autologous combination of fresh effector and target cells.

Cancer patients have traditionally been treated with some combination of surgery, chemotherapeutic agents and radiation. Although some patients have responded in varying degrees to these treatment modalities, others are relatively unresponsive. Immunotherapy was proposed as a fourth treatment modality, and a number of clinical trials have been performed by using a variety of BRM, including recombinant cytokines such as interferon (IFN), interleukin (IL)-2, and tumor necrosis factor (TNF)-α and adoptively transferred cytolytic effector cells such as lymphokine-activated killer (LAK) cells and activated tumor-infiltrating lymphocytes (TIL). Data obtained in such clinical trials showed response rates of 5% to 25% [9, 15, 23]. The response rates for most protocols have not been markedly increased by alterations of doses and schedules of treatment. It remains unclear why a subset of patients responds to a given treatment modality, while the majority remain unresponsive. It is thus important to search for some immunological parameter or tumor characteristic that could be used prospectively to predict patient response to a given treatment modality and/or to provide definitive answers regarding the mechanisms responsible for an antitumor response.

Here we describe the biological and clinical significance of ATK activity in human cancer patients and also show that ATK induction therapy may improve the clinical outcome in patients who naturally have no such potential.

ATK activity of T lymphocytes and NK cells

Autologous combination of fresh effector and target cells have been used in cytotoxicity tests of human cancer patients: Blood samples were obtained from each patient prior to anesthesia for surgery, and non-adherent lymphocytes consisting mainly of T lymphocytes and NK cells were used as effectors [2–5, 10–12, 21–22, 24–33]. Tumor cells with more than 90% purity and viability were isolated from tumor specimens obtained from cancer patients at the time of surgery by mechanical and enzymatic treatment, followed by centrifugation on discontinuous three-step Percoll and two-step Ficoll-Hypaque gradients and by adherence to plastic surfaces in our laboratory. Blood lymphocytes of cancer patients lyse autologous, freshly isolated tumor cells in a short-term.(4–18 h) cytotoxicity assay [2, 3, 11, 24, 30–33]. Positive reactions have been recorded with blood samples of 5% to 80% cancer patients, depending on the type and stage of tumors studied, and the institution performing the study. Tumor types studied include adenocarcinoma or squamous cell carcinoma of the lung, breast, stomach, liver, colon, ovary, uterus, melanoma, head and neck, rhabdomyosarcoma, leiomyosarcoma, osteosarcoma, malignant lymphoma and leukemia.

ATK activity is mediated by T lymphocytes and/or LGL in human cancer patients. $CD3^+$ T lymphocytes produce autologous tumor-restricted cytotoxic effects when patients have localized neoplasms [3, 26]. A number of T cell clones with restricted ATK activity were established from cancer patients [4, 7–9, 19]. Most T cells recognize foreign antigens only when these antigens are associated on cell surfaces with the membrane glycoproteins encoded by genes in MHC. In fact, the mediation of ATK by T lymphocytes usually

requires both interaction with self MHC determinants and the appropriate antigenic determinant [2, 7–9]. However, there exists exceptions in that some cytotoxic T lymphocytes recognize the antigenic structures on autologous tumor cells either without association with MHC gene products or in association with relatively non–polymorphic antigens [2, 7]. In addition, T cells lysed autologous tumor cells expressing no detectable MHC glycoproteins in considerable numbers of cancer patients. Tumor cells freshly isolated from cancer patients lack the expression of MHC class I and/or II molecules on their surface in 10–50% tumor samples. It seems thus likely that a proportion of human tumor cells share determinants that are different from normal MHC molecules and are recognized by autologous T lymphocytes.

Fresh human tumor cells were previously shown to be largely refractory to lysis by blood lymphocytes of normal donors and suggested to express no or minimal NK-relevant structures [13]. Our recent evidence, however, indicates that $CD3^-$ LGL of cancer patients also lyse autologous fresh tumor cells [3–5, 12, 22]. The effector cells released a novel cytotoxic factor, termed ATKF that produces lytic effects on autologous and allogeneic fresh human tumor cells, without affecting nonmalignant cell types [16, 17]. ATKF is also produced by T lymphocytes in response to autologous tumor cells. All of the data taken together indicate that the mediation of ATK activity is performed by $CD4^-CD8^+$ or $CD4^+CD8^-$ T lymphocytes and/or $CD3^-$ LGL in patients with localized neoplasms and primarily by LGL in patients with advanced and metastatic disease [11, 16]. NK cells preferentially kill target cells without MHC molecules on their surface. In the ATK system, however, there is no correlation between MHC expression of tumor cells and their susceptibility to lysis by autologous LGL. Thus, the ATK system does not always follow the general rule established in studies on cytotoxicity and recognition of allogeneic tumor cells and tumor cell lines. It should be noted that some $CD3^-$ LGL clones established express autologous tumor-restricted lysis, without killing of allogeneic tumor cells or NK prototype target K562 [2]. In this respect a subset of NK cells are shown to recognize and kill allogeneic target cells through interaction with MHC molecules [34, 35].

An absence of leukocyte function-associated antigen 1 (LFA-1) on the cell surface has been proposed to be one mechanism of tumor escape from immunosurveillance [37]. Freshly isolated human tumor cells expressed varying levels of the intercellular adhesion molecule 1 (ICAM-1; CD54) on their surface [2, 36]. ICAM-1 was more frequently seen in squamous cell carcinoma than in adenocarcinoma. Both $CD54^+$ and $CD54^-$ fractions of adenocarcinoma were lysed by autologous T cells and LGL, while ATK lymphocytes killed preferentially $CD54^+$ tumor cells in squamous cell carcinomas [38–40]. ATK effector cells expressed varying levels of adhesion molecules, including CD11a, CD11b, CD11c, CD18 and CD54 on their surface. These adhesion molecules, however, was not always involved in ATK. Of interest is the finding that YTA-1 monoclonal antibodies (mAb) reactive with a novel epitope of a chain of LFA-1 activates lymphocytes to express ATK through protein tyrosine phosphorylation [41, 42].

Table 3. ATK mechanisms.

1.	Directed motility of ATK lymphocytes toward autologous tumor cells.
2.	Recognition of the tumor; MHC-dependent or -independent.
3.	Tight binding to the target through interaction with LFA-1.
4.	Activation of lytic machinery of ATK lymphocytes; 59 kD and 70 kD protein tyrosine phosphorylation.
5.	Release of a novel cytotoxic factor. termed ATKF.
6.	Incorporation of ATKF into tumor cells.
7.	DNA fragmentation and death of the target.

Much effort has been made to analyze the mechanisms involved in the interaction between lymphocytes and autologous tumor cells. It is now possible to define a sequence of evens which appears to be necessary for the lytic process (Table 3) [16, 17, 39–41]. The lysis of fresh human tumor cells by ATK lymphocytes includes:

1) The ATK lymphocyte moves toward an autologous tumor cell and recognizes the tumor, which is either dependent or independent on MHC molecules expressed on tumor cells.
2) After recognition of autologous tumor cells, the ATK lymphocytes tightly bound to the target cells through interaction with LFA-1 molecules.
3) The lytic machinery of ATK lymphocytes is activated by bound autologous tumor cells, in which 59 kD and 70 kD protein tyrosine is phospohorylated.
4) The tyrosine phosphorylation of p59 and p70 caused ATK lymphocytes to release a novel cytotoxic factor. termed ATKF.

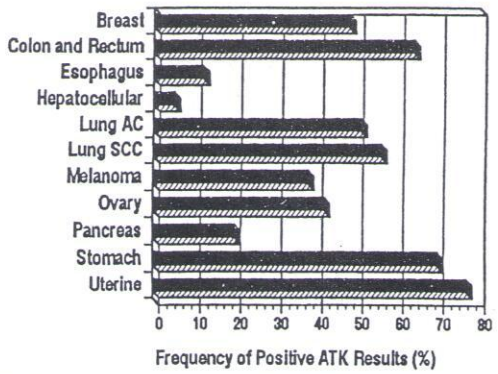

Fig. 1. Frequencies of positive results in ATK tests in human cancer. Blood lymphocytes from patients with various cancers were tested for ATK activity at the time of surgery.

5) Finally, the released ATKF is incorporated into tumor cells, resulting in DNA fragmentation and death of the targets.

Clinical significance of ATK

The above evidence suggests that the ATK system may be important in the control of tumor growth and eventual rejection of tumor. To obtain direct evidence that ATK cells play an essential role in immune defense against tumor, we have tested ATK activity and compared it with other immunological and biological parameters in more than 2,000 patients with various stages and types of malignant neoplasms [2, 4, 38–40]. The malignancies studied include adenocarcinoma or squamous cell carcinoma of the lung, breast, stomach, liver, colon, ovary, uterus, and melanoma. Blood lymphocytes from cancer patients demonstrated varying levels of ATK activity. Significant cytotoxicity was documented in 5–80% cancer patients, depending on tumor types and stages. The lack of ATK activity is unlikely to be due to the inability of certain cancer patients to mount immunologic response, since tumor cells of these patients could be lysed by killer cells that were activated by IL-2, the streptococcal preparation OK432, protein-bound polysaccharide, b-1–3–D-glucan sizofiran and Japanese herbal medicine Kampo in most cases [13, 30, 21].

The frequency of positive results in ATK tests was relatively high (60–80%) in stomach and breast cancer patients whose prognosis is relatively good, while it was extremely low (5–20%) in patients with hepatocellular carcinoma, esophageal cancer and pancreas cancer whose prognosis is bad (Fig. 1). The number of ATK-positive samples decreased when patients developed metastasis. In patients with localized neoplasms, T-cells and/or LGL exhibited ATK, while it was mediated primarily by LGL when patients had metastases [26]. T cells of metastatic cancer patients could recognize autologous tumor cells but fail to express ATK function since LGL suppressed the acquisition of ATK potential by T cells [26]. Results in ATK tests were not indicated by the clinical and pathological TNM stage while the primary tumor was localized [29, 38].

The prognostic significance of ATK tests was proposed by previous studies on lung cancer and osteosarcoma [32, 33]: The patients with ATK activity survived longer than those without the activity. It was, however, unclear whether ATK cells play a role in host defense against tumor since no statistical analysis of survival curves was performed, the follow-up period was short and other immunological functions were not concomitantly tested in those studies. It might be possible that patients with ATK activity have better general immune status and thus survive longer than those without the activity.

Direct evidence of the biological and clinical significance of the ATK system could be obtained only in studies that meet the following criteria:

1) Cancer patients should receive complete curative surgery.
2) Clinical parameters that must be assessed prospectively include performance status of the ECOG (Eastern Cooperative Oncology Group) performance status scale, age, sex, weight loss, other organ system diseases, and use of medications.
3) Pathological examinations should perform TNM classification and confirm the absence of tumor cells in the margins of tumor resections.
4) No adjuvant anticancer therapy may be performed after surgery. When local or distant recurrence developed, patients would receive chemotherapy, radiation therapy, or biological therapy.
5) Patients should be followed for up to at least 5 years.
6) Survival curves should be estimated by the Kaplan-Meier method and adequate statistical analysis such as the Cox-Mantel and generalized Wilcoxon test.
7) Other immunological tests should be concomitantly performed.
8) Other factors that have been reported to influence the prognosis of cancer patients should also be measured, including abnormal expression of oncogenes

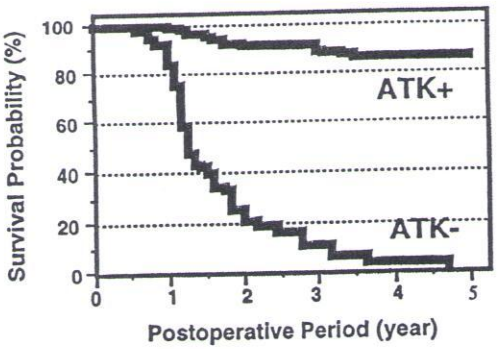

Fig. 2. Curves for postoperative tumor-free interval of ATK-positive and -negative patients. Blood lymphocytes from each patients were tested for ATK activity at the time of surgery, and patients were retrospectively evaluated for disease-free interval after 5 years of follow-up after curative operation. Tumor-free survival curves were estimated by Kaplan-Meier analysis.

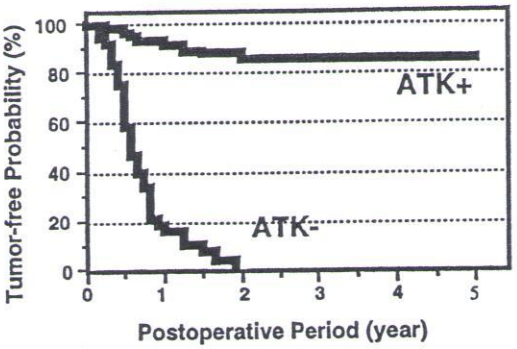

Fig. 3. Curves for postoperative total survival of ATK-positive and -negative patients. Blood lymphocytes from each patients were tested for ATK activity at the time of surgery, and patients were retrospectively evaluated for total survival after 5 years of follow-up after curative operation. Total survival curves were estimated by Kaplan-Meier analysis.

and tumor suppressor genes and DNA patterns of tumor cells.

We have performed such studies and published the results in elsewhere [2, 4, 22, 29, 38–40]. Patients with primary, localized tumors received complete curative surgery, were tested for ATK functions and were retrospectively evaluated for postoperative tumor-free and total survival. It is of interest and importance to note that more than 80% of cancer patients whose blood lymphocytes expressed ATK activity at the time of surgery have remained disease-free and alive more than 5 years after the operation (Fig. 2, 3). The other small proportions of patients with ATK activity, however, developed metastases by 2 years and died by 5 years. In contrast, all patients with no demonstrable ATK activity relapsed within 2 years and died within 5 years. The differences observed in curves for postoperative disease-free interval and long-term survival for patients with or without ATK activity estimated by Kaplan-Meier analysis were statistically highly significant according to the Cox-Mantel and generalized Wilcoxon test. The correlation coefficient for ATK and postoperative clinical course was also high. The data strongly indicate that the potential of blood lymphocytes to kill autologous fresh tumor cells, tested at the time of surgery, may predict good prognosis of patients with primary localized tumors. The results also suggest that the measurement of ATK function at the time of surgery in cancer patients will provide valuable information for the probability of disease recurrence [23]. Also, it is predicted that ATK activity may be a useful prognostic factor for patient selection for BRM trials and that treatment protocols that effectively induce ATK activity would be beneficial to cancer patients.

Although all patients received curative surgery, only those with ATK ability are free from tumor and alive for more than 5 years. Updated clinical data indicate that none of the patients who have remained tumor-free for the observation period of 5 years developed recurrence and died after that period. Patients with ATK activity might have better background factors and thus survive longer than those without the activity. There are, however, no differences in background factors including performance status, clinical and pathological TNM classification, age or sex between ATK-positive and -negative groups. These results suggest that ATK activity may represent an independent prognostic parameter. All patients without ATK activity developed recurrence and died within 5 years, showing that a negative result in ATK tests definitively indicates a poor prognosis. Our results suggest that ATK lymphocytes may be the main effectors in the host defense system against tumor. The test, however, has no absolute prognostic value, since some patients with short disease-free interval and short-term survival also had positive results. The immunological control may not be operative for some types of cancer. Taken together, these data are consistent with the hypothesis that the immune system may play a beneficial role in the eventual tumor rejection in at least some patients.

One may argue that cancer patients with lymphocytes having ATK activity have better general immune status and thus survive longer than those without this activity. The data of concomitant tests of oth-

er immunological functions, however, have demonstrated that ATK activity is not correlated with T cell proliferation induced by autologous tumor cells, LGL-mediated NK activity against K562, mitogenic response, autologous and allogeneic mixed lymphocyte reactions, and production of cytokines including IFN, IL-1, IL-2 and TNF [29, 38]. It is thus evident that the absence of ATK activity does not reflect impaired general immunity of cancer patients. In retrospective evaluation, the presence or absence of immunological functions other than ATK did not correlate with postoperative disease-free and total survival Similarly, no association was demonstrated between NK activity and postoperative prognosis in melanoma [43], sarcoma [32] and lung cancer patients [33]. By contrast, the prognostic value of NK activity was documented in patients with breast cancer [44] and with head and neck cancer [45]. The difference might be due to the tumor types studied. The data that we obtained in patients with breast cancer, however, argue against the prognostic significance of NK activity [29].

The abnormal expression of oncogenes and tumor suppressor genes has been implicated as critical factors involved in the development of tumor [46]. Prognostic significance of oncogenes and tumor suppressor genes is currently controversial [47, 48]. A recent report showed correlation between survival and amplification of oncogene-coamplification units in breast cancer patients [49]. In our study, however, the abnormal expression of oncogenes, including c-*myc*, c-*fos*, c-*erbB*-2 and K-*ras*, and/or tumor suppressor genes such as *RB* and p53 in tumor cells was independent on ATK activity and does not correlate with the postoperative clinical course of cancer patients [2, 29, 38]. Also, ATK activity did not correlate with the DNA pattern (aneuploid or diploid) of tumor cells [38].

The correlation of ATK activity with long-term survival of patients has been observed in various tumor types, including lung cancer, breast cancer, stomach cancer, hepatocellular carcinoma, colon cancer, sarcoma and melanoma. Thus, this correlation does not appear to be unique to the pathophysiology of some cancer but may represent a prognostic factor for many cancers. These studies include only patients who had primary localized tumors and underwent curative surgery and whose tumor specimens were suitable for cytotoxicity. Such specimens have usually been obtained in approximately 30–80% of patients with solid tumors of different origins. Thus, the possibility that the strong correlation of ATK activity and clinical course may be true only for a proportion of cancer patients could not be ruled out.

ATK induction therapy and its clinical significance

The strong correlation of ATK activity with long-term, tumor-free status is consistent with the hypothesis that the immune system may play a beneficial role in the eventual rejection of tumors [29]. The evidence also suggests that these cytotoxic cells may play some critical role in the interaction between cancer patients and their own tumor. Thus, basing therapeutic strategies on the activity of other effector cell types may be misleading [40, 50, 51]. The biological effects of BRM have widely been assessed for the ability to augment NK activity and induce LAK activity, which is mainly derived from NK cells [14]. However, since there was no correlation between NK activity and survival, conclusions drawn from such parameters may be irrelevant for the function of ATK cells and clinical course of cancer patients. Thus, the optimization of BRM doses and schedules for different effector activities may not translate to the best conditions for ATK activity.

The conventional design and performance of cancer biotherapy might be irrelevant. Adoptive immunotherapy with various types of activated killer cells such as LAK and TIL has been beneficial only for a subset of patients with melanoma and renal cell carcinoma [9, 15, 18, 52]. The actual mechanism responsible for the observed antitumor effects of adoptively transferred these cytotoxic cells is not yet understood. This may be a result of their cytolytic activity or due to their helper function to activate other cell components involved in the inhibition of tumor growth and metastasis. The evidence that ATK activity, but not NK activity, correlates with prognosis favors the latter explanation that adoptively transferred LAK cells may function indirectly. TIL therapy is based on the concept that TIL sensitized *in vivo* by autologous tumor cells could become differentiated *in vitro* to mature ATK lymphocytes by restimulation with autologous tumor cells and IL-2 and that those effector cells might produce therapeutic benefit. Since TIL were stimulated to proliferate *in vitro* with autologous tumor cells, they may be more analogous to the putatively non-prognostic lymphocytes that respond in autologous mixed lymphocyte-tumor cultures than to the predictive ATK lymphocytes [29]. In fact, TIL express little or no ATK activity, though they have good proliferative capacity to autologous tumor

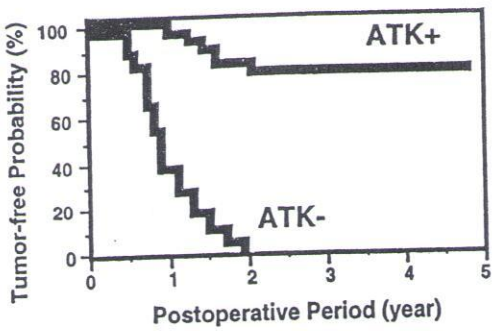

Fig. 4. ATK induction therapy and prolongation of tumor-free survival. Prior to curative operation patients with various types of cancer such as hepatocellular carcinoma, stomach cancer and breast cancer received BRM that had been proved to induce ATKF in *in vitro* tests. Patients were evaluated for disease-free survival after curative operation.

cells [4]. In addition, ATK activity of TIL was not associated with disease-free and total survival, which is in contrast with that of blood lymphocytes. Thus, important effector cells may actually be found in the blood rather than in tumor tissues.

It is of clinical and biological importance to demonstrate that *in vivo* induction of ATK activity could inhibit the tumor growth and prolong the survival of patients who naturally have no such potential. To this end, patients with localized hepatocellular carcinoma, lung cancer, stomach cancer and breast cancer have been treated with BRM prior to operation and evaluated for the postoperative clinical course. Approximately 50–90% patients who originally had no ATK activity responded to the biotherapy to acquire the activity by the time of surgery [22, 4]. Vast majority of cancer patients who were induced to have ATK function by the therapy have remained disease-free and alive more than 5 years after the operation (Fig. 4). Five year survival was approximately 70% for hepatocellular carcinoma, 85% for breast cancer and more than 90% for stomach cancer. In contrast, all patients with no ATK activity in spite of the therapy developed local and/or distant recurrence by 2 years and died by 5 years. As is the case with spontaneous ATK activity, BRM-induced ATK activity strongly correlated to postoperative clinical course of patients who received biotherapy prior to operation. By contrast, BRM-induced other immune functions did not correlate with long-term survival of the patients. These data may imply that the biotherapy with ATK-inducting effects may produce clinical benefit to cancer patients who naturally have no ATK activity. It should, however, be determined why a subset of patients respond to a given BRM to acquire ATK activity and long-term survival, while others do not. The failure of ATK induction may be due to the responsiveness of lymphocytes to a given BRM or to the resistance of tumor cells to BRM-activated autologous killer lymphocytes.

The biological and immunological significance of BRM-induced ATK activity has also been established in patients with metastatic cancer patients whose clinical course could not be improved by conventional approaches. Previous studies demonstrated that local administration of BRM such as bacterial preparations and glucan to patients with carcinomatous pleural or peritoneal effusions results in an induction of ATK activity [21]. It should be noted that the induction of ATK activity is strongly associated with a reduction or complete disappearance of tumor cells in the effusions. The observed antitumor effect did not parallel with other immunological parameters. Patients with other types of far advanced cancer received adoptive cellular immunotherapy by the use of blood lymphocytes that had previously been activated with BRM. BRM that are used to stimulate lymphocytes are selected by their ability to induce the protein tyrosine phosphorylation of p59 and p70 and production of ATKF *in vitro*. Cancer patients received intravenous administration of 10×10^{12} activated ATK lymphocytes once to 3 times a week [42]. The ATK induction therapy has induced complete disappearance or partial regression of tumor, resulting in prolongation of total survival time in the majority of cancer patients;. The clinical effect has been documented in patients with advanced stages of lung cancer, stomach cancer, breast cancer, and sarcomas. The induced ATK activity has positively correlated with prolonged survival time, while such a correlation is not observed with other parameters.

Questions would then arise about the relevance of different BRM-induced effector cells to antitumor response. Various agents have been administered to induce cytotoxic lymphocytes capable of lysing autologous tumor cells, including IL-2 [13], anti-CD3 antibody [53], bacterial preparations [21], polysaccharides [30], and b-glucan [4], and Japanese herbal medicine Kampo (unpublished observation). Different effector cells with different cytolytic functions have been induced. Some type of cytolytic cells such as LAK cells recognize and kill nonmalignant normal cell types in addition to autologous tumor cells, which may be one reason of the limited efficacy of LAK therapy. In fact administration of IL-2 prior to surgery is not found to prolong disease-free survival in cancer patients. In oth

er procedures blood lymphocytes from cancer patients are stimulated *in vitro* with anti-CD3 antibody and grown with IL-2, and then they are adoptively transferred to autologous cancer patients in phase I clinical trials [53]. Stimulation of T cells and LGL with YTA-1 mAb are found to induce ATK activity, which may be relevant to spontaneous ATK activity [42].

Conclusion

The overall results presented in this review demonstrate that positive ATK activity at the time of surgery predicts a favorable clinical course in patients who have primary localized solid tumor and receive curative operation. The strong correlation of ATK activity with disease-free interval and total survival (1) indicates that ATK activity is a meaningful prognostic indicator and (2) provides evidence for immunological control of tumor growth and metastasis. Based on the potential biological significance of ATK activity, clinical trials have been conducted to determine whether the induction of ATK activity could improve the clinical outcome in patients who naturally have no such potential. The preliminary data indicate that the presence of both natural and induced ATK activity is strongly associated with long-term survival. Thus, considerable emphasis should be placed on a strategy that induces ATK activity *in vivo*. Such an approach may provide a new focus for cancer immunotherapy.

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