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Review

Biological significance of autologous tumor-killing activity and its induction therapy

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Introduction

At the beginning of this century the thesis that host defense forces may prevent neoplastic cells from developing into tumors was formulated. If such forces did not exist, the incidence of tumor occurrence would be much higher. In fact, the increased incidence of malignant tumors observed in patients receiving continuous immunosuppressive treatment following organ transplantation, or that of Kaposi's sarcoma in AIDS (acquired immune deficiency syndrome) patients, is often cited in support of the concept that immune surveillance mechanisms operate against human malignant diseases. In response to foreign antigens, T cells with specific receptors undergo clonal selection, proliferation, and differentiation. One of the major functions of these effector T cells is to recognize cells carrying the antigen and eliminate them. Accumulated evidence, however, indicates that T-cell-mediated immunity is mainly important for protection against tumors induced by oncogenic viruses and not against spontaneous tumors or chemical-carcinogen-induced tumors [27]. A variety of components of natural and adaptive immunity, including T cells, natural killer (NK) cells, K cells, monocytes/macrophages, and granulocytes play a role in immune resistance against tumor [22, 39], which leads to the following predictions. (a) Tumor cells express antigens or structures that are recognized by one or more effector types. In fact, the same human tumor cells were recognized by autologous T cells, NK cells and monocytes/macrophages [40, 49, 55]. (b) Tumor cells are susceptible to cytotoxicity or growth inhibition mediated by one or more effector mechanisms. Indeed, fresh human tumor cells are killed by autologous T cells [6, 7, 14, 17, 52], large granular lymphocytes (LGL) [38, 42], lymphokine-activated killer (LAK) cells [10, 11, 28], and soluble cytotoxic factors [36, 47]. (c) One or more of the relevant effector cells should enter the site of tumor growth. A variety of effector cell types have been observed within or around the tumor site in cancer patients, and they demonstrate cytotoxicity against autologous tumor cells in vitro [14, 29, 31, 41]. (d) Potentiation of relevant effector mechanisms may decrease the incidence of tumor or metastasis [43, 48, 49]. (e) Suppression of relevant effector mechanisms may increase the incidence of tumor. (f) Restoration of impaired effector functions may reduce the incidence of tumor. Thus, the actual version of the immunological surveillance hypothesis involves the assumption that malignant cells express membrane structures or antigens that are recognized by one or more members of the immune system, and that these various effectors could eliminate transformed cells.

In vitro cell-mediated cytotoxicity is considered to be one of the expressions of host immune defense mechanisms against a tumor [24]. Demonstration of in vitro destruction of human tumor cells by various components, particularly cells of the immune system, has had a strong impact on the concept of immune surveillance. The outcome of the cytotoxicity assay depends on several factors: the immunological history of lymphocyte donors, the activation profile and composition of lymphocyte populations, the previous treatment of effector cells, and the characteristics of tumor cells, all of which may represent a balance of the cytolytic potential of various effector cells, their subsets and antibodies with affinity of target recognition sites and other factors contributing to innate target-cell susceptibility. Several types of effector cells with different mechanisms of interaction may contribute to cell lysis and even within one experiment different subsets may act simultaneously on the very same target. Because of the complexity of the immune system, the results obtained in vitro do not necessarily reflect in vivo events. Accordingly, absence of in vitro tumor cell lysis in a patient does not necessarily rule out the existence of an immune response. Conversely, the in vitro demonstration of cytotoxic lymphocytes, does not necessarily indicate that tumor cell destruction occurs in vivo.

Most studies on cell-mediated cytotoxicity against tumor have been performed by the use of in vitro cultured

cell lines as targets. It is difficult, however, to interpret the data on cytotoxicity against cultured tumor cell lines, since in vitro culture of tumor cells may alter their surface antigenic properties and susceptibility to cell-mediated lysis through several mechanisms [39, 48]: (a) selective growth of tumor cells that are adapted to in vitro culture conditions; (b) a loss of putative tumor-associated and/or major histocompatibility complex (MHC) antigens that have previously been expressed on tumor cells in vitro; (c) an appearance of a new antigen(s) and/or structure(s) that does not exist in vivo. Few investigators, however, have used freshly isolated tumor cells as targets in human systems, which may be mainly due to the difficulty of obtaining fresh tumor cells with high purity and good viability. Enzyme and mechanical treatment may alter the surface characteristics of fresh tumor cells and cause cell damage, some of the contaminating stromal cells may serve as targets, and the contamination with lymphoid cells and monocytes/macrophages may influence cytotoxicity. Recent development of technology has enabled us to obtain fresh human tumor cells with high purity and good viability. In addition, the autologous combination of effector and target cells is of critical importance in the search of tumor antigenicity since T lymphocytes are thought to recognize tumor target cells through interaction of T cell antigen receptor (TCR)/CD3 complex and MHC/HLA antigens [6, 14, 17, 29]. By contrast, the cytotoxicity of NK cells is not restricted by antigens encoded by the MHC gene [34]. Thus, for a better understanding of the cytolytic function of lymphocytes against tumor in human cancer patients, it is desirable to perform a cytotoxicity assay with an autologous combination of fresh, untreated effector and target cells.

Autologous mixed lymphocyte/tumor culture (AMLTC) has been used to investigate the recognition by lymphocytes of autologous tumor cells in mixed culture [40, 41, 45]. If cancer patients are sensitized against tumor-associated antigens, the tumor cells are expected to stimulate the proliferation of the lymphocytes in vitro. Indeed, T lymphocytes are induced to proliferate in response to autologous tumor cells, while the proliferation is not observed or is minimal after stimulation with allogeneic tumor cells. In addition, the T-cell subset that responds to autologous tumor cells has been found recently to differ from the subset that is reactive to autologous non-malignant non-T cells [44, 45]. Taken together, these results indicate that a tumor-specific event may be observed in the AMLTC.

Cancer patients have traditionally been treated with some combination of surgery, chemotherapeutic agents, and radiation. While some patients respond in varyious degrees to these treatment modalities, others are relatively unresponsive. Immunotherapy has been proposed as a fourth modality of cancer treatment, and a number of clinical trials have been performed by the use of a variety of biological response modifiers (BRM), including recombinant cytokines and adoptively transferred cytotoxic effector cells such as LAK cells and activated tumor-infiltrating lymphocytes (TIL). Such clinical studies demonstrated response rates of 5%–25%, depending on the BRM used and the type of cancer patients treated [9, 28, 29]. The response rates for most protocols have not been markedly elevated by alterations in doses and schedules of treatment. Thus, it remains unclear why a subset of patients responds to a given treatment modality, while the majority remain unresponsive. It is 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 autologous tumor killing (ATK) activity in cancer patients and also show that an in vivo induction of ATK activity prior to surgery by administration of BRM may improve the clinical outcome in patients who naturally have no such potential.

Autologous tumor killing (ATK)

In cytotoxicity studies autologous combinations of untreated effector and fresh target cells have been used in cancer patients: blood samples have been obtained from each patient prior to anesthesia for surgery, and non-adherent lymphocytes, consisting mainly of T lymphocytes (60%–95%) and LGL (5%–40%) have been used as effectors [34–49]. Tumor cells were isolated from tumor specimens obtained from cancer patients at the time of surgery by enzymatic treatment (DNase, collagenase, hyarulonidase), followed by centrifugation on discontinuous three-step Percoll gradients and two-step Ficoll/Hypaque gradients and by adherence to plastic surfaces. The resulting tumor-enriched fraction contained usually more than 90% tumor cells that were more than 90% viable in our laboratory. Unfractionated blood lymphocytes lyse tumor cells freshly isolated from the same patients in a short-term (4–18 h) cytotoxicity assay [2, 16, 18, 37–39, 49–53]. Various positive reactions have been recorded with blood samples from 5%–70% cancer patients, depending on the types of tumors studied, the stages of tumor, and the institution performing the study. Tumor types studied include adenocarcinoma or squamous cell carcinoma of the lung, breast, stomach, liver, colon, ovary, uterus and melanoma, head and neck cancer, malignant lymphoma and leukemia. Highly purified tumor cell populations with good viability could not be obtained from solid neoplasms without enzyme treatment, which may affect target cell sensitivity. In another series of experiments the enzyme effect was avoided by the use of tumor cells from effusions. Blood lymphocytes of patients with carcinomatous pleural effusions exhibited cytotoxicity against such tumor cells in approximately 25% of cases [37, 38]. Frequencies and levels of cytotoxicity observed with effusion types of tumors were similar to those seen with solid neoplasms [37, 39]. When the impact of enzymes, especially trypsin, was tested, the susceptibility of fresh human tumor cells was altered in either a positive or negative way [39]. When compared with blood lymphocytes, tumor-infiltrating lymphocytes were less or equally effective in killing of autologous tumor cells.

In initial studies blood lymphocytes of patients with localized tumor killed autologous fresh tumor cells in

30%–50% cases, while reactions were recorded for allogeneic tumor cells in fewer than 10% of cases [53]. Since cytotoxicity by fresh T cells is restricted to the MHC-gene-encoded proteins, these data may indicate that T lymphocytes are responsible for lysis of autologous fresh tumor cells. In fact, small $CD3^+$ T lymphocytes obtained from cancer patients have cytotoxic effects on autologous tumor cells when patients have localized neoplasms [40, 51]. Furthermore, a number of T cell clones with restricted ATK activity have been established from the blood and tumor tissues [7, 14, 17, 31, 49]. It is thus evident that ATK activity is mediated by T lymphocytes. Most T cells recognize foreign antigens only when these antigens are associated on cell surfaces with the membrane glycoproteins encoded by genes in MHC. The MHC molecules are thought to serve as primitive antigen-binding receptors that help each class of foreign antigen to activate the appropriate type of T cells. In fact, the lysis of fresh human tumor cells by autologous T lymphocytes requires both interaction with self MHC determinants and with the appropriate antigenic determinant in the majority of cases [7, 14, 17, 26, 39]. However, there exist 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 [7, 39]. In addition, T cells lysed autologous tumor cells carrying no detectable class I and class II MHC glycoproteins in considerable numbers of cancer patients. Furthermore, $CD3^+CD4^-CD8^+$ T cells killed autologous tumor cells without MHC class I molecules and $CD3^+CD4^+CD8^-$ T cells lysed autologous tumor cells without MHC class II molecules [39]. Analysis by the use of monoclonal antibodies (mAb) has revealed that freshly isolated tumor cells from human cancer patients lack the expression of MHC class I and/or class II molecules on their surface in 10%–50% tumor samples, depending on tumor types. In addition, blocking of MHC class I antigen expression by anti-MHC class I mAb did not always abrogate their lysis by autologous T cells, though the treatment abolished the ATK sensitivity in the majority of cases. The data may indicate that a proportion of human tumor cells share determinants that are different from normal MHC molecules and are recognized by autologous T lymphocytes.

It was previously suggested that freshly isolated human tumor cells express no or minimal NK-relevant structures since freshly derived human tumor cell suspensions were largely refractory to lysis by untreated blood lymphocytes of normal donors [10]. The results from recent studies of cell population and single-cell levels, however, clearly indicate that $CD3^-CD16^+$ LGL of cancer patients also lyse autologous, freshly isolated tumor cells [38, 42, 48]. In addition, the effector cells released a novel cytotoxic factor with lytic effects on autologous and allogeneic fresh human tumor cells, without affecting non-malignant normal cells [36, 47]. All of the data taken together indicate that the mediation of ATK activity is performed by $CD3^+CD4^-CD8^+$ or $CD3^+CD4^+CD8^-$ T lymphocytes and/or $CD3^-$ LGL in patients with localized neoplasms and primarily by LGL in patients with advanced and metastatic disease [36, 38]. NK cells have been shown to preferentially recognize and lyse target cells expressing no MHC molecules. However, there was no correlation between MHC expression of tumor cells and their susceptibility to lysis by autologous LGL. Furthermore, the induction of MHC class I molecules by treatment of tumor cells by interferon (IFN) γ and tumor necrosis factor (TNF) α did not cause them to become resistant to lysis by autologous LGL in the majority of cases. 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 that were established by repeated stimulation with autologous tumor cells express autologous tumor-restricted lysis, without killing allogeneic fresh tumor cells or the NK prototype target K562. In this respect recent in vivo and in vitro evidence indicates that a subset of NK cells recognize and kill allogeneic target cells through interaction with MHC molecules [4, 8].

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 [5]. Flow cytometry analysis revealed that freshly isolated human tumor cells expressed various levels of the intercellular-adhesion molecule 1 (ICAM-1; CD54) on their surface [15, 39]. The expression of ICAM-1 was more frequently observed with squamous cell carcinoma than with adenocarcinoma. Both $CD54^+$ and $CD54^-$ fractions of adenocarcinoma were lysed by autologous T cells and LGL, while ATK effector cells killed preferentially $CD54^+$ squamous cell carcinomas. ATK effector cells expressed various levels of adhesion molecules, including CD11a (α chain of LFA-1), CD11b (α chain of Mac-1), CD11c (α chain of p150/95), CD18 (β chain of the LFA-1 family) and CD54 on their surface. Treatment of ATK effector cells with mAb against these adhesion molecules, however, did not always abrogate cytotoxicity against autologous tumor cells. Of interest is the finding that the YTA-1 mAb, reactive with a novel epitope of the α chain of LFA-1 molecules, activates blood- and tumor-infiltrating lymphocytes to express ATK activity. Other anti-LFA-1 mAb so far reported had no such activity. Thus, the LFA-1 molecule transduces important signals involved in ATK and the ATK effector cells kill autologous tumor cells either through LFA-1/ICAM-1 interactions or independently.

Biological significance of ATK

As stated above, the ATK system may be important in the control of tumor growth. It is of importance to obtain direct evidence that ATK effector cells play an essential role in immune resistance against tumor. One approach is to test lymphocyte reactivity against autologous, freshly isolated tumor cells by the use of cytotoxicity and proliferation assays and compare it with other immunological and biological parameters in various stages of human cancer. We have performed such studies in more than 1500 patients with various types of malignant neoplasms, including adenocarcinoma or squamous cell carcinoma of the lung, breast, stomach, liver, colon, ovary and uterus, and mela-

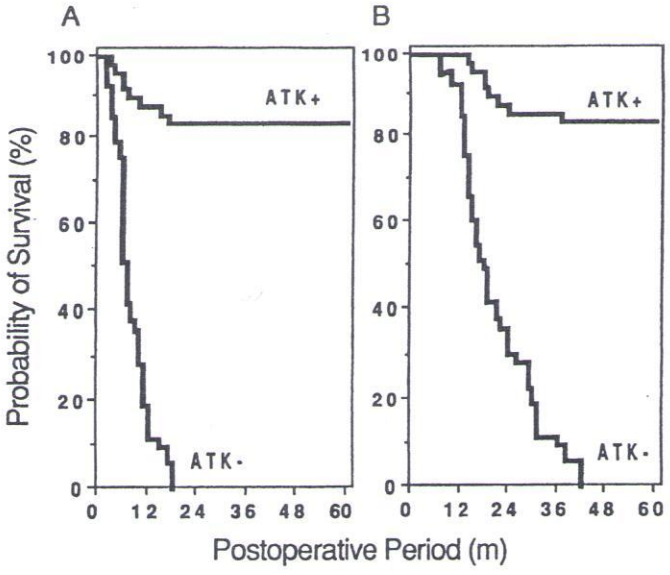

Fig. 1. Curves for postoperative disease-free interval (**A**) and total survival (**B**) of 98 autologous-tumor-killing(ATK)-positive patients and 95 ATK-negative patients. Blood lymphocytes from each patient were tested for ATK activity at the time of surgery, and patients were retrospectively evaluated for disease-free and total survival after 60 months of follow-up after curative operation. Tumor-free and total survival was estimated by Kaplan-Meier analysis

noma. At the time of surgery, blood lymphocytes from patients with a variety of primary localized solid neoplasms demonstrated various levels of ATK activity. Significant cytotoxicity has been observed in 5%–70% 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 an immunological response, since fresh tumor target preparations from these patients could be lysed in most cases by killer cells that were induced by interleukin-2 (IL-2), the streptococcal preparation OK432, protein-bound polysaccharide, or the β1-3D-glucan sizofiran [10, 16, 43].

The frequency of positive results in ATK tests was high (60%–70%) in patients with stomach or breast cancer whose prognosis is known to be relatively good, while it was extremely low (5%) in hepatocellular carcinoma patients whose prognosis is bad. The number of samples that were positive for ATK activity decreased when patients developed metastasis. In patients with localized neoplasms, $CD3^-$ LGL and/or T cells expressed ATK activity, while it was mediated primarily by LGL when patients developed metastases [35]. This is not a result of the failure of T cells to recognize autologous tumor cells since T cells of patients with metastatic tumor responded in vitro to autologous tumor cells by proliferating and developing autologous tumor-specific cytotoxicity in the absence of LGL. LGL of patients with metastatic cancer were found to suppress the induction of ATK potential in the AMLTC, which may explain the low frequency of T cells with ATK activity in these patients [35]. Results in ATK tests were not indicated by the clinical and pathological TNM stage while the primary tumor was localized [46]. These results suggest that ATK activity may have biological significance.

The prognostic significance of ATK tests in cancer patients has previously been proposed by studies in which patients with lung cancer or osteosarcoma were tested for ATK activity at the time of surgery and were retrospectively evaluated for the postoperative clinical course [50, 51]: the patients whose blood lymphocytes exhibited ATK activity survived longer than those without the activity. It was, however, unclear whether ATK effector cells play an essential role in host defense against tumor since, in those studies, no statistical analysis of survival curves was performed, and the follow-up period was short. In addition, other immunological functions were not concomitantly tested. It might be possible that patients with lymphocytes having ATK activity have better general immune status and thus survive longer than those without the activity.

To obtain direct evidence of the biological role of ATK systems it is essential to perform studies that meet the following criteria. (a) Cancer patients should receive complete curative surgery. (b) Clinical parameters to be assessed prospectively must include performance status on the ECOG (Eastern Cooperative Oncology Group) scale, age, sex, weight loss, diseases of other organ systems, and use of medications. (c) Pathological examinations should perform a TNM classification and confirm the absence of tumor cells in the margins of tumor resections. (d) No adjuvant anticancer therapy should be performed after surgery; when local or distant recurrence developed, patients receive chemotherapy, radiation therapy, or biological therapy. (e) Patients should be followed for at least 5 years. (f) Survival curves should be estimated by the Kaplan-Meier method and adequate statistical analysis such as the Cox-Mantel test and generalized Wilcoxon test. (g) Other immunological tests should be concomitantly performed. (h) Other factors that have been reported to influence the prognosis of cancer patients should also be measured, including amplification and/or deletion of oncogenes and suppressor genes, and DNA patterns (aneuploid or diploid) of tumor cells.

Such studies have been performed and the results have recently been published [39, 46, 48, 49]. Patients with primary, localized tumors received complete curative surgery, were tested for ATK functions at the time of surgery and were retrospectively evaluated for postoperative tumor-free and total survival time. The patients who are

alive have had follow-up for at least 5 years. It is of interest 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. 1). The other patients with ATK activity, however, developed metastases by 2 years and died within 4 years. In contrast, all the patients with no demonstrable ATK activity relapsed within 2 years and died within 4 years. When the disease-free interval and total survival time were estimated by Kaplan-Meier analysis, the differences observed in curves for postoperative survival (disease-free interval and long-term survival) for patients with or without ATK activity were statistically highly significant according to the Cox-Mantel test and the generalized Wilcoxon test. The correlation coefficient for ATK and the 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 represent a good prognosis for 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 on the probability of disease recurrence [23]. The data also predict 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 are alive after more than 5 years. Whereas it might be said that patients will relapse and die after the 5-year observation period, this possibility can be ruled out since updated clinical data indicate that none of the patients who have remained tumor-free for the observation period developed recurrence and died after that period. Patients with ATK activity might have a better performance status and TNM classification 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 groups that were positive for ATK activity and those that were negative. These results suggest that the measurement of 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 immunological defense system against growth and metastasis of tumor. The test, however, has no absolute prognostic value, since some patients with a short disease-free interval and short total survival also had positive results. Because some patients without ATK activity have other normal immunological functions, the immunological control may not be operative for some types of cancer. 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.

It is of importance to exclude the possibility that cancer patients with lymphocytes having ATK activity have better general immune status and thus survive longer than those without this activity, by concomitantly testing other immunological functions. The data obtained in concomitant tests have demonstrated that ATK activity is not correlated with T cell proliferation induced by autologous tumor cells in mixed cultures or LGL-mediated NK activity against the NK prototype target K562. In fact, ATK is mediated by heterogeneous populations: LGL in 35% of the patients, T lymphocytes in an additional 30%, and both types of lymphocytes in the remaining 35% [39]. In addition, there were no correlations between ATK activity and other immune functions, including mitogenic response, autologous and allogeneic mixed-lymphocyte reactions, and production of interferon, IL-1 and IL-2 [46]. 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 NK cell activity, T cell reaction in AMLTC, or other immunological functions did not correlate with either postoperative disease-free interval or total survival. Similarly, no association has been demonstrated between NK cell activity and postoperative prognosis in melanoma patients [13], sarcoma patients [50], and lung carcinoma patients [51]. The findings of other studies, however, supported the prognostic value of NK activity in patients with breast cancer [21] and with head and neck cancer [30]. The difference might be due to the tumor types or cancer patients studied. The data we obtained in patients with breast cancer, however, argue against the prognostic significance of NK activity [39]. In other studies leukocyte-dependent antibody activity has been reported to have prognostic significance in melanoma patients [12].

The acquisition of oncogenes as well as the loss of tumor-suppressor genes has been implicated as a critical factor involved in the development of tumor [19]. The clinical prognostic significance of oncogenes and tumor-suppressor genes is currently controversial [1, 32]. A recent report has shown a correlation between survival and amplification of oncogene-coamplification units in breast cancer patients [33]. In our study, however, ATK activity was independent of oncogene amplification of tumor cells, including c-*myc*, c-*fos*, c-*erbB*-2 and K-*ras*. In addition, deletion of tumor-suppressor genes such as *RB* and the p53 gene of tumor cells was not associated with their sensitivity to ATK effector cells [39], and the abnormal expression of oncogenes and/or tumor-suppressor genes does not correlate with the postoperative clinical course of cancer patients. Also, ATK activity did not correlate with the DNA pattern (aneuploid or diploid) of tumor target cells.

The correlation of ATK activity with long-term survival of patients has been observed not only in lung cancer but also in other types of cancer, including breast cancer, stomach cancer, hepatocellular carcinoma, colon cancer, and sarcoma. Thus, this correlation does not appear to be unique to the pathophysiology of some cancers but may represent a prognostic factor for many cancers. It should be noted, however, that 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%–70% of patients with solid tumors of different origins, depending on tumor types and institutions studied. Thus, the possibility that the strong correlation of ATK activity and postoperative clinical course may be true

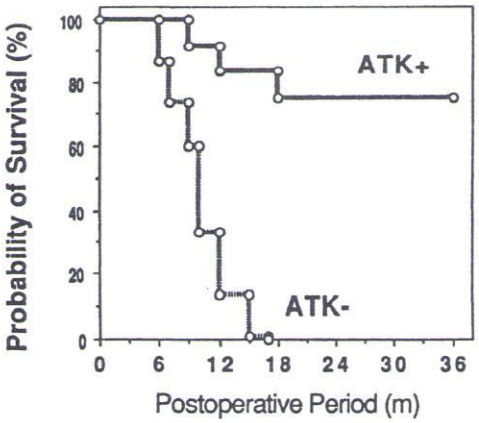

Fig. 2. Induction of ATK activity by biological therapy and prolongation of tumor-free survival. Patients with hepatocellular carcinoma received biological therapy such as OK432 or sizofiran before curative operation. Approximately 50% of patients acquired ATK activity at the time of surgery. Patients were evaluated for disease-free survival after curative operation, and survival was estimated by Kaplan-Meier analysis

only for a proportion of cancer patients can not be ruled out. Further studies must be performed to confirm the biological significance of ATK activity.

Induction of ATK activity and its biological significance

The strong correlation of blood 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 at least some tumors. The evidence also suggests that these cytotoxic cells may play some critical role in the interaction between cancer patients and their own tumors. Thus, basing therapeutic strategies on the activity of other effector cell types may be misleading. The biological effects of BRM have widely been assessed for the ability to augment NK cell activity and induce LAK cell activity, which is mainly derived from NK cells. However, since there was no positive correlation between NK cell activity, tumor-free interval and total survival, conclusions drawn from such parameters may be irrelevant for the function of ATK effector cells and the clinical course of cancer patients. Thus, the optimization of BRM doses and schedules for different effector activities may not translate into the best conditions for ATK activity.

The conventional design and performance of cancer treatment approaches with BRM might be irrelevant. Adoptive cellular immunotherapy with the use of various types of activated cytolytic cells such as LAK and TIL has been beneficial only for a subset of patients with melanoma and renal cell carcinoma [9, 20, 25, 28, 29]. However, the actual mechanism responsible for the observed antitumor effects of adoptively transferring these cytotoxic cells is not yet understood. This may be a result of their cytolytic activity or be due to their helper function in activating some other cell component involved in the inhibition of tumor regrowth and metastasis. The evidence that ATK activity, but not NK activity, correlates strongly with patients' prognosis favors the latter explanation, that adoptively transferred, activated NK cells may function indirectly. The adoptive immunotherapy with activated TIL is based on the concept that TIL sensitized in vivo by autologous tumor cells could become differentiated in vitro to mature ATK lymphocytes by re-stimulation with autologous tumor cells and IL-2 and that those effector cells might be involved in the therapeutic benefit observed with this treatment. 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 AMLTC than to the predictive ATK lymphocytes [46]. In fact, TIL are found to express little or no ATK activity, while they have good proliferative capacity to autologous tumor cells in the mixed culture [49]. In addition, the ATK activity of TIL was not associated with long-term survival and disease-free interval, which is in contrast with 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. Previous studies have demonstrated that intrapleural administration of the streptococcal preparation OK432 to patients with carcinomatous pleural effusions results in an induction of ATK activity [43]. 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. Again, the observed antitumor effect has not been correlated with other immunological parameters, such as augmentation of NK cell activity and AMLTC reactions. Similar effects have been observed with other BRM including the β1-3D-glucan sizofiran.

Further studies have been performed to ascertain whether in vivo induction of ATK activity prior to surgery could inhibit the tumor regrowth and metastasis formation after curative operation and prolong the postoperative clinical course in patients who naturally have no such potential. Patients with localized hepatocellular carcinoma have been treated with these BRM prior to curative operation and evaluated for the postoperative clinical course. Approximately 50% patients who naturally had no blood lymphocytes with ATK activity responded to the biological therapy to acquire ATK activity by the time of surgery [48, 49]. The induced ATK activity has been maintained for at least 4 weeks after the operation by repeated administration of the agent. The patients who are alive have had follow-up for at least 3 years. No adjuvant anticancer therapy was performed after surgery. When patients relapsed, they received chemotherapy, radiation therapy and/or immunotherapy. More than 75% of cancer patients who are induced by the therapy to express ATK activity at the time of surgery have remained disease-free and alive more than 3 years after the operation (Fig. 2). The other patients with BRM-induced ATK activity, however, developed metastases within 2 years. In contrast, all of the patients with no demonstrable ATK activity in spite of the biological therapy developed local and/or distant recurrence within 1 year and had died by 2 years. As is the case with spontaneous

ATK activity, there are strong correlations of the presence of ATK activity at the time of surgery with the postoperative clinical course in patients with hepatocellular carcinoma. Other immunological parameters were also altered by the biological therapy. In contrast to ATK activity, however, BRM-augmented NK cell activity and other immune functions did not correlate with the long-term survival of the patients. These data may imply that the biological therapy with ATK-inducing effects before surgery may produce clinical benefits for cancer patients who naturally have no ATK activity.

Based on the results and possible implications of these preliminary studies, further studies must be performed to confirm a biological significance of the BRM-induced ATK activity. It should be determined whether the correlation of induced ATK activity with long-term survival represents a prognostic factor for various types of cancer or is unique to the pathophysiology of hepatocellular carcinoma. Such a correlation has been observed with breast cancer and stomach cancer (unpublished observation), supporting the former possibility. It should also be determined why a subset of patients respond to a given BRM and acquire ATK activity, resulting in 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 lymphocytes.

Questions then arise about the relevance of different BRM-induced effector cells to antitumor response. Various agents have been administered to induce cytolytic cells in vitro capable of lysing autologous tumor cells, including IL-2 [10], anti-CD3 antibody [3], bacterial preparations [43], polysaccharides [16], and β-glucan [49]. Different effector cells with different cytolytic functions have been induced. Some types of cytolytic cells, such as LAK cells, recognize and kill non-malignant 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 preliminary studies. In other procedures blood lymphocytes from cancer patients are stimulated in vitro with anti-CD3 antibody and grown with IL-2 [3], and then they are adoptively transferred to autologous cancer patients in phase I clinical trials. Stimulation of $CD3^+CD8^+$ T cells with YTA-1 antibody directed against a novel epitope of LFA-1 molecules is found to induce autologous tumor-restricted cytolytic activity independently of IL-2/IL-2 receptor systems, which may be relevent to spontaneous ATK activity.

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 (a) indicates that ATK activity is a meaningful prognostic indicator and (b) provides evidence for immunological control of tumor growth and metastasis. According to these data, it is unlikely that cancer patients who remain tumor-free after 5 years of follow-up will develop recurrence or die from the disease. Although there is no direct evidence that ATK effector cells play a critical role in regression of tumor and prevention of tumor regrowth, the lack of ATK activity in patients who relapsed and died after surgery may not result from factors related to their poor performance status since no differences have been observed in background factors between ATK-positive and -negative groups. The prognostic value of ATK activity in patients with documented metastatic tumors has not been established yet. In this respect, however, the induction of ATK activity by BRM has positively correlated with prolonged survival time, while such a correlation is not observed with other parameters such as NK cells or LAK cell activity.

Based on the possible biological significance of ATK activity, clinical trials have been conducted to determine whether the induction of ATK activity before surgery by administration of BRM 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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