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Activation of T Lymphocytes for the Adoptive Immunotherapy of Cancer

Jeffrey J. Sussman, MD, Suyu Shu, PhD, Vernon K. Sondak, MD, and Alfred E. Chang, MD

Background: Adoptive immunotherapy of malignancy involves the passive transfer of antitumor-reactive cells into a host in order to mediate tumor regression. Based on animal models, the transfer of immune lymphoid cells can eradicate widely disseminated tumors and establish long-term systemic immunity. Critical for successful adoptive immunotherapy is the ability to isolate large numbers of immune cells. For clinical therapy, it will require the development of in vitro methods to promote the sensitization and propagation of tumor-reactive cells. However, this is a formidable task since human cancers are postulated to be poorly immunogenic because of their spontaneous origins.

Results: Human lymphoid cells for ex vivo activation and subsequent adoptive transfer have been derived from different sources, including peripheral blood, tumor, and lymph nodes. Peripheral blood lymphocytes can be incubated with interleukin 2 to generate lymphokine-activated killer (LAK) cells, which nonspecifically lyse autologous and allogeneic tumor cells in vitro. LAK cell therapy represented the earliest attempt to treat advanced human cancers, with encouraging results documented in patients with renal cell cancer and melanoma. From that experience, the use of more immunologically specific cellular agents with potentially greater therapeutic efficacy has been investigated. One approach uses tumor-infiltrating lymphocytes, which have been characterized experimentally to be more specific in tumor reactivity compared with LAK cells. Other techniques have involved the use of lymphoid cells derived from lymph nodes draining tumors or primed by tumor vaccines. In vitro activation of these cells with tumor antigen or anti-CD3 monoclonal antibody results in the generation of T cells that mediate the rejection of poorly immunogenic tumors in animal studies. These alternate methods are currently being evaluated in clinical studies.

Conclusions: Experimentally, cellular therapy is a potent method to eradicate progressive tumors. Initial clinical studies have demonstrated that this form of therapy is technically feasible and can result in meaningful antitumor responses. Advances in this area will require improved methods to sensitize, isolate, and expand tumor-reactive T cells for adoptive transfer.

Key Words: Adoptive immunotherapy—Antitumor-reactive cells—Lymphoid cells—Tumor regression.

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From the Division of Surgical Oncology, University of Michigan, Ann Arbor, Michigan, USA.

Address correspondence and reprint requests to Alfred E. Chang, Division of Surgical Oncology, 1500 E. Medical Center Drive, Ann Arbor, MI 48109-0331, USA.

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Ever since experimental tumors were discovered to express unique antigens subject to tumor-specific transplantation rejection, immunotherapeutic methods to treat cancer have been intensely studied. Unlike the success seen in clinical transplantation, stemming from the possibility of suppressing the immune system, attempts at enhancing immuno-

logic function to specifically reject progressively growing human tumors have been less straightforward. Immunotherapy of cancer can be broadly classified into two major categories: active and passive immunotherapy. Active immunotherapeutic approaches rely upon the stimulation of endogenous host immune elements to react against tumor antigens. By contrast, passive immunotherapy involves the administration of agents that have been characterized to have antitumor reactivity, in order to mediate a therapeutic effect in the host. These approaches have been established initially in animal models and have helped define basic principles of the host's immune response against progressive tumor.

One of the earliest attempts in cancer immunotherapy was active immunotherapy with nonspecific immune stimulants. This approach was based on the hypothesis that nonspecific immune stimulation of the host would result in an increased reactivity to putative tumor antigens on growing cancers. Bacterial and pharmacological immune stimulants, including *Bacillus Calmette-Guérin* (BCG), *Corynebacterium parvum* (*C. parvum*), *Nocardia rubra,* and levamisole, were employed in the 1970s to induce systemic immunity. However, these clinical trials failed to show consistent therapeutic benefits in cancer patients treated with these nonspecific immune stimulants (1). More recently, the availability of cytokines has afforded the clinician the ability to treat metastatic disease by modulation of the host's immune response. The cytokine that has been studied the most is interleukin 2 (IL-2); it has been documented to have limited therapeutic efficacy against melanoma and renal cell carcinomas. From several studies, the response rates with IL-2 therapy for these cancers are between 10 and 20% (2–5).

Another form of active immunotherapy that has been investigated for many years is the use of tumor vaccines. This method has also been referred to as active specific immunotherapy. A potential obstacle to this approach is the weak immunogenicity of human cancers by virtue of their spontaneous origins. Moreover, the host may be partly immunosuppressed from the presence of progressively growing tumor. To help overcome these obstacles, irradiated tumor cells were often mixed with an immune adjuvant, such as BCG, or otherwise manipulated to become more effective at stimulating a host response. More recently, genetic modification of tumor cells has been reported to increase tumor immunogenicity and may serve to produce more potent tumor vaccines. In animal models, transfection of tumors with various cytokine genes, allogeneic major histocompatibility complex antigens or immune costimulatory molecules have been shown to increase the host immune response against unmodified, parental tumor cells (6–13). The efficacy of gene-modified tumor cells in clinical active specific immunotherapy is currently being examined in clinical trials.

The other major category of immunotherapies is passive immunotherapy. The two most common elements of passive immunotherapy are tumor-reactive antibodies and cellular reagents. The use of specific antibodies for cancer therapy is also referred to as "passive immunization"; it has been explored for many years with somewhat disappointing results (14–16). However, efforts to produce bifunctional conjugates of toxins or radiochemicals coupled to antibodies specific for tumor cells have been encouraging (17,18). Evidence suggesting the existence of common tumor antigens lends impetus to this type of approach, but clinical utility still must be confirmed (19,20). This review will focus on the different approaches of passive cellular therapy that have been established in animal studies and their application in clinical trials.

ADOPTIVE IMMUNOTHERAPY

Immune rejection of tumors has been demonstrated to be T cell mediated, with antibodies playing an insignificant role in this phenomenon. Historically, the passive transfer of T cells, but not serum, from an immunized animal to a naive host conferred protective immunity to subsequent tumor challenges. While such immune T cells clearly mediate a protective antitumor effect, they also show therapeutic utility in the treatment of established tumors (21,22). This form of therapy has also been termed "adoptive immunotherapy". Important principles of adoptive immunotherapy established from animal studies are:

1. Syngeneic (or autologous) immune cells are more efficacious than allogeneic and xenogeneic cells.
2. The therapeutic efficacy of immune cells is dependent on the number of cells transferred.
3. Tumor-induced suppression of the host immune response to tumor has been demonstrated.
4. Tumors vary in degree of immunogenicity.
5. Lymphoid cells derived from tumor-immune an-

imals can be cultured in vitro and still retain antitumor activity when passively transferred in vivo.
6. Concomitant administration of IL-2 can enhance the in vivo therapeutic activity of cultured immune cells.

Using inbred strains of mice, it was noted that successful adoptive immunotherapy of established tumor required the transfer of large numbers of immune lymphocytes derived from syngeneic donor mice that were hyperimmunized against the same tumor. Obviously, this approach cannot be directly applied to the treatment of human cancers since genetically identical individuals are not available as a source of immune lymphocytes. Therefore, one critical problem in the development of clinical adoptive immunotherapy has been finding a source of immune cells that can be obtained from the cancer patient. Unfortunately, cancer patients probably represent a preselected population who lack the ability to adequately respond to their own tumors. Moreover, animal studies have shown the existence of tumor-induced immunosuppression that may interfere with the sensitization of lymphoid cells or their ability to mediate antitumor effects (23–25). Therefore, it became apparent that the generation of large numbers of functional T cells with specific antitumor reactivity was going to be dependent upon designing in vitro methods to further sensitize and expand lymphoid cells from the tumor-bearing host. In this regard, a succession of lymphocyte activation strategies have been examined in an attempt to generate therapeutic cellular agents. Included in this list are lymphokine-activated killer (LAK) cells, tumor-infiltrating lymphocytes (TILs), in vitro sensitized lymphocytes, and anti-CD3/IL-2 activated lymphocytes.

Lymphokine-activated killer cells

The incubation of lymphoid cells in high concentrations of IL-2 results in the generation of LAK cells, a phenomenon described by Grimm et al. (26). LAK cells are characterized as non-T, non-B lymphoid cells that mediate the nonspecific in vitro cytolysis of autologous as well as allogeneic tumor cells. This phenomenon is distinct from that characteristic of conventional cytotoxic T lymphocytes (CTLs), where sensitized T cells mediate immunologically specific tumor cytolysis in vitro. The specific cytotoxicity manifested by CTLs requires that they share the same class I major histocompatibility (MHC) antigens as the tumor to which they are sensitized, whereas the nonspecific tumor cytotoxicity mediated by LAK cells does not have such MHC restriction. Nevertheless, LAK cells were found to have in vivo therapeutic efficacy in the treatment of micrometastatic tumor (27,28). Mice with established 3-day pulmonary metastases had significant tumor regression when treated with LAK cells administered in conjunction with IL-2 (Fig. 1). These investigations have shown that the in vivo antitumor effects of LAK cells were nonspecific and highly dependent on the concomitant administration of IL-2.

Based on these experimental observations, Rosenberg and co-workers at the National Cancer Institute (NCI) conducted the first human trial of adoptive immunotherapy with autologous LAK cells and IL-2, which was reported in 1985 (29). In that study, 25 patients with advanced cancer (i.e., melanoma and renal cell, colon, and lung cancer) were treated with $\leq 1.8 \times 10^{11}$ LAK cells generated from peripheral blood lymphocytes (PBLs) obtained through several leukaphereses. They were also given bolus infusions of IL-2 at maximum tolerated doses. Eleven of the patients experienced measurable tumor regressions: one complete and 10 partial. This study heralded the feasibility of activating large numbers of lymphocytes ex vivo for adoptive immunotherapy of human cancer. Because this early experience indicated that patients with melanoma and renal cell cancer were most responsive, subsequent studies mainly focused on pa-

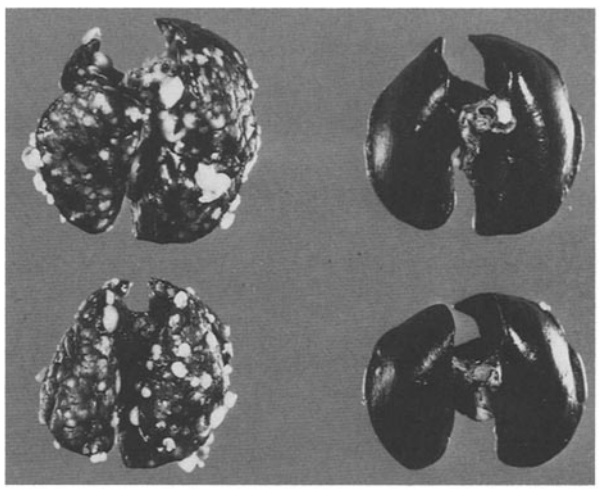

FIG. 1. Adoptive immunotherapy of 3-day MCA 105 pulmonary micrometastases with LAK cells and IL-2. Whole lungs were harvested 14 days after i.v. tumor inoculation and insufflated with India ink via the trachea to allow enumeration of the white lung tumors. Lungs on the left are from control mice without treatment, and lungs on the right are from animals treated with LAK cells and nontherapeutic doses of IL-2 (28).

tients with these tumor histologies. Table 1 summarizes the results of using LAK cells plus IL-2 in the treatment of advanced melanoma and renal cell cancer at several institutions (30–40). In 190 patients with melanoma, there was a response rate (complete and partial) of 16%. Among 198 patients with renal cell cancer, the response rate was 22%. In these two populations of patients, sites of tumor regression included liver, lung, bone, skin subcutaneous tissue, and lymph nodes. It is noteworthy that if tumor regressed at one site in a patient, it usually was associated with tumor regression at all sites of disease (30). In addition, a large proportion of patients who experienced complete responses maintained these responses over a long period of time.

Analyses of factors associated with tumor responses from LAK and IL-2 therapy have turned up no consistent findings. The number of LAK cells infused, the in vitro lytic activity of the LAK cells, and the total amount of IL-2 administered were not predictive of achieving a tumor response. The toxicities associated with the therapy were mainly due to the effects of IL-2. Multiple organ toxicity associated with IL-2 infusion can be attributed mostly to induction of a capillary leak syndrome, marked lymphocytic infiltration within visceral organs, and elaboration of other cytokines in response to IL-2 (41). The severity of these toxicities were clearly associated with the cumulative amount of IL-2 administered. Fortunately, these toxicities were quickly reversible once IL-2 therapy was discontinued.

Since high-dose IL-2 alone was shown to have antitumor efficacy in patients with advanced melanoma and renal cell cancer, it was important to determine whether the addition of LAK cells offered any improved therapeutic benefit. In a multiinstitutional trial, 167 patients with advanced melanoma and renal cell cancer were randomized to receive LAK cells plus IL-2 or IL-2 only (42). No significant difference between the treatment arms was observed. In melanoma patients, the response rates for LAK/IL-2 and IL-2 were 12% and 16%, respectively. In renal cell cancer patients, the response rates for LAK/IL-2 and IL-2 were 13% and 8%, respectively. In a similar prospective, randomized trial conducted by Rosenberg et al. at the NCI, the response rates in patients with melanoma and renal cell cancer were not statistically different for LAK/IL-2 versus IL-2 therapy (43). However, there was a trend toward more complete responses in patients treated with LAK/IL-2 versus IL-2 only, and there was a trend toward improved survival in patients with melanoma randomized to receive LAK/IL-2 compared with IL-2 alone. These LAK cell trials

TABLE 1. *Efficacy of LAK cells and IL-2 in the therapy of renal cell carcinoma and melanoma*

Author	No. of patients	No. of LAK cells[a]	IL-2 infusion	Tumor responses		
				CR	PR	CR + PR
Melanoma						
Rosenberg (30)	34	7.9×10^{10}	Bolus	3	3	6
West et al. (31)	10	$6.8–9.1 \times 10^{10}$	CI	0	5	5
Schoof et al. (32)	9	4.3×10^{10}	Bolus	1	4	5
Thompson et al. (33)	8	$3.4–4.3 \times 10^{10}$	Bolus/CI	0	0	0
Paciucci et al. (34)	5	3.4×10^{10}	CI	0	1	1
Dutcher et al. (35)	36	8.9×10^{10}	Bolus	1	5	6
Bar et al. (36)	55	8.3×10^{10}	CI	1	6	7
Dutcher et al. (37)	33	1.6×10^{11}	CI	0	1	
Total	190			6	25	31 (16%)
Renal cell cancer						
Rosenberg (30)	54	7.9×10^{10}	Bolus	7	10	17
West et al. (31)	6	$6.8–9.1 \times 10^{10}$	CI	0	3	3
Schoof et al. (32)	10	4.3×10^{10}	Bolus	0	5	5
Thompson et al. (33)	8	$3.4–4.3 \times 10^{10}$	Bolus/CI	1	0	1
Paciucci et al. (34)	32	$4.9–6.1 \times 10^{10}$	CI	2	5	7
Wang et al. (38)[b]	9	3.4×10^{10}	CI	0	1	1
Fisher et al. (39)	32	7.0×10^{10}	Bolus	2	3	5
Parkinson et al. (40)	47	9.2×10^{11}	CI	2	2	2
Total	198			14	29	43 (22%)

CI, continuous infusion; CR, complete response (regression of all evaluable tumor); PR, partial response (≥50% regression of all evaluable tumor).

[a] Represents mean or median number of cells.

[b] Modified LAK generated by periodate and IL-2.

stimulated efforts to find more potent cellular agents. From animal studies, it was evident that the therapeutic efficacy of passive cellular therapy was enhanced as the cellular specificity toward the individual neoplasm increased.

Tumor-infiltrating lymphocytes

One such population of tumor-specific T cells are TILs. TILs are generated by the culture of dissociated cells from solid tumors in high concentrations of IL-2. Rosenberg et al. found that adoptive immunotherapy with TILs significantly prolonged survival in mice with advanced pulmonary metastases that were refractory to LAK cells (44). Optimal treatment included cyclophosphamide, followed by the administration of TILs and IL-2 (Fig. 2). The role of cyclophosphamide in this model is not clearly understood. It may have acted to lower tumor-induced immunosuppression or it may have served as a cytoreductive agent. In contrast to LAK cells, TILs have been shown to have relatively more specific immunoreactivity against the tumor of origin in in vitro cytotoxicity assays. This tumor specificity was associated with a 50- to 100-fold increased efficacy in the reduction of lung metastases when compared with LAK cells (45).

In patients with metastatic melanoma, adoptive immunotherapy with autologous TILs and IL-2 with or without cyclophosphamide resulted in a 38% combined partial and complete response rate at the NCI (46). Compared with the LAK/IL-2 trials in melanoma, this rate suggested that TIL therapy may be more efficacious in the treatment of melanoma. However, problems with the consistent culture and expansion of lymphocytes from resected tumor along with the labor-intensive laboratory procedures necessary to generate large numbers of TILs for therapy have limited widespread clinical application of this technique. There has been very little clinical experience outside the NCI with the use of large numbers ($>10^{11}$) of TILs for therapy. In renal cell cancer, Bukowski et al. reported no responses in 18 patients treated with large numbers of TILs and escalating doses of IL-2 (47). This finding may reflect that certain tumor histologies are not amenable to the generation of therapeutically effective TILs. It is evident that the optimal conditions for TIL culture and expansion as well as its clinical applications need to be further defined.

In vitro sensitized cells

We have developed a technique of promoting the stimulation and growth of tumor-specific lymphocytes called in vitro sensitization (IVS) (48,49). This technique involves the culture of lymphocytes with irradiated tumor-stimulator cells in the presence of low concentrations of IL-2. In animal studies, we examined the antitumor reactivity of lymphoid cells from normal and tumor-bearing mice that had been cultured by the IVS technique. We found that freshly harvested tumor-draining lymph node (TDLN) cells had no therapeutic effect in adoptive immunotherapy experiments; however, after IVS culture, this lymphocyte population was found to be very effective at mediating the regression of 3-day pulmonary metastases (Table 2). Since in vitro sensitized cells derived from the spleens of normal mice did not mediate antitumor effects, the induc-

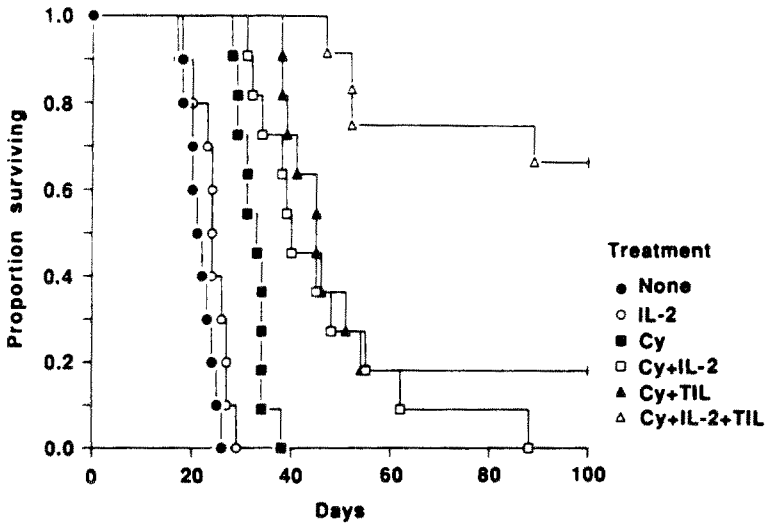

FIG. 2. Adoptive immunotherapy of advanced MC-38 pulmonary metastases with TIL. Treatment of mice with advanced pulmonary metastases from the immunogenic MC-38 colon adenocarcinoma. This figure summarizes two experiments in which treatment was begun 12 and 14 days, respectively, after i.v.injection of tumor cells. Mice received cyclophosphamide (Cy) at 100 mg/kg i.v. 6 h before TIL (2–2.4 × 10^7 cells i.v.) and IL-2 (20,000 U i.p. every 8 h for 5 days) were given (44).

TABLE 2. *Adoptive immunotherapy of MCA 105 pulmonary metastases with fresh noncultured or in vitro sensitized cells from mice bearing progressive MCA 105 tumors*

Source of lymphoid cells[a]	Mean no. of pulmonary metastases (SEM)[b]	
	Fresh lymphoid cells	In vitro sensitized lymphoid cells
—	136 (33)	171 (51)
Normal spleens	127 (37)	91 (46)
Immune spleens	2 (1)[c]	1 (1)[c]
Tumor-draining lymph nodes	86 (26)	2 (1)[c]

Modified from Shu et al. (48).

[a] Lymphoid cells from spleens or lymph nodes of normal or MCA 105 immune mice or mice bearing 14-day s.c. MCA 105 tumor.

[b] Fresh lymphoid cells (1.5×10^8 spleen cells or 6×10^7 lymph node cells) or in vitro sensitized lymphoid cells (2×10^7) were given i.v. to mice with established 3-day MCA 105 pulmonary metastases. Animals receiving in vitro sensitized cells also were given 7,500 U IL-2 i.p. twice daily for 3 days after cell transfer. Pulmonary metastases were counted on day 14.

[c] $p < 0.05$ compared with groups of mice not treated with cells.

tion of tumor-sensitized cells in TDLN cells must have occurred during progressive tumor growth. Although lacking functional antitumor reactivity when initially removed, these "preeffector" cells differentiated during IVS culture to become therapeutically active effector cells. Further studies showed that in vitro sensitized cells derived from TDLN cells were effective in the treatment of advanced metastatic disease in the absence of cyclophosphamide (Fig. 3) (50).

As alluded to previously, the immunogenicity of a tumor may be a significant factor in its ability to generate antitumor reactive T cells. For example, TILs derived from poorly immunogenic animal tumors have been therapeutically ineffective in adoptive immunotherapy experiments (45). Using these same tumors, we also observed that TDLN cells cultured in IVS did not result in the generation of functionally active effector cells. These observations underscore the problem with the immunotherapy of poorly immunogenic tumors, which more closely resemble human cancers. Nevertheless, we were able to develop an alternative approach to elicit preeffector cells in the host with the use of the bacterial immunoadjuvant, *C. parvum*. We found that inoculations of tumor cells admixed with *C. parvum* induced the sensitization of preeffector cells in the draining lymph nodes (LN) of mice several days later. The subsequent culture of these "primed" LN cells led to the generation of potent effector cells, as assessed in adoptive immunotherapy experiments (51,52). Further studies confirmed that the phenotype of in vitro sensitized effector lymphocytes was predominantly $CD8^+$ and that $CD4^+$ cells were required only as a source of IL-2 during in vitro differentiation. This fact was verified by in vivo depletion techniques of specific T-cell subpopulations before TDLN harvest, where $CD4^+$ function could be replaced by IL-2 in the IVS culture. In adoptive immunotherapy experiments, we found that only $CD8^+$ cells were necessary to mediate the regression of pulmonary metastases and that the exogenous administration of IL-2 enhanced this effect.

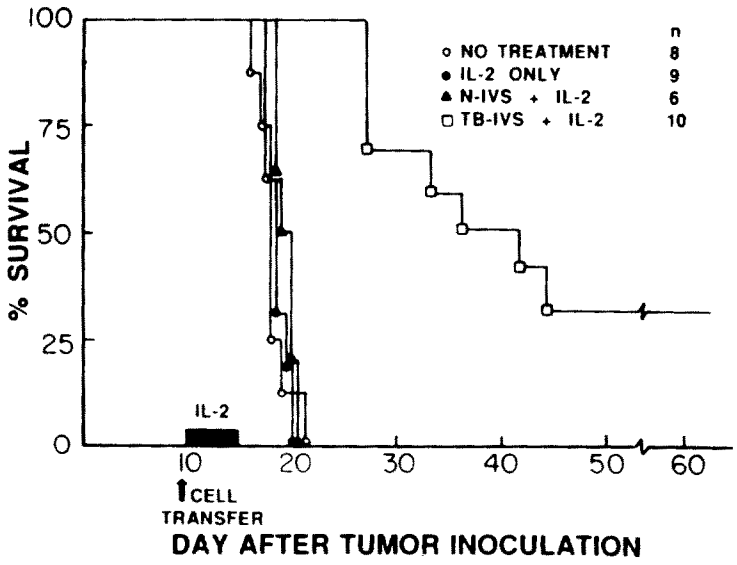

FIG. 3. Survival benefit of adoptive immunotherapy of 10-day established pulmonary MCA 105 metastases with in vitro sensitized lymph node cells generated from mice bearing 14-day intrafootpad tumors (TB-IVS). IVS lymph node cells from normal mice (N-IVS) served as control. Each mouse received 3.0×10^7 cells i.v. and exogenous IL-2 i.p. (7,500 U/0.5 ml Hanks' balanced salt solution, twice a day for 5 days) (50).

The IVS technique has been applied to humans with advanced malignancy (53). An autologous tumor vaccine comprising irradiated tumor cells admixed with the bacterial adjuvant BCG was used to develop vaccine-primed LN cells for subsequent IVS culture. After differentiation and expansion by the IVS method, effector cells were transferred to patients with concomitant administration of IL-2. We found that the majority of patients who received in vitro sensitized cells developed cellular reactivity to autologous tumor, as assessed by delayed-type hypersensitivity reactivity, compared with a cohort of patients who were treated by tumor vaccination and IL-2 administration only. Despite the transfer of cutaneous antitumor reactivity with IVS cells, there was limited metastatic tumor regression. This finding may be related to insufficient numbers of transferred in vitro sensitized cells (median 7×10^9 per patient), which was due in part to the limited quantity of autologous tumor stimulator cells available for IVS culture. To help overcome the need for tumor during the in vitro activation procedure, alternative strategies to activate preeffector cells have been developed by our laboratory.

Anti-CD3/IL-2 activated lymphocytes

A prerequisite to specifically activate sensitized T cells in culture requires the binding of antigen to the T-cell receptor/CD3 complex expressed on the cell surface. Hence, we needed to identify an alternative method to actively sensitize T cells in the absence of antigen. A monoclonal antibody to the T-cell receptor/CD3 complex (anti-CD3) has been found to cause T-cell proliferation and cytokine production in a fashion similar to that seen with T-cell-receptor binding to antigen (54). From these observations, we examined the activation of murine TDLN cells with anti-CD3 instead of tumor cells, followed by culture of these cells in low concentrations of IL-2. This activation procedure resulted in a five- to 10-fold expansion of TDLN cells. The adoptive transfer of these anti-CD3/IL-2 activated cells mediated the regression of established pulmonary metastases in murine models (55,56). Although T-cell activation by the anti-CD3 antibody was polyclonal, the therapeutic effects mediated by these cells was immunologically specific, with the specificity determined by the tumor that stimulated the draining lymph node.

We have characterized the requirements for optimal anti-CD3/IL-2 culture conditions in animal models (Table 3). The activation sequence, amount of monoclonal antibody, and IL-2 concentrations were all of critical importance for the development of therapeutically active effector cells. The effector cells generated by the IVS and anti-CD3 activation methods were similar in their therapeutic effect on a per cell basis. However, they differed in other aspects and probably represent either different effector cell populations or different stages of differentiation of the same effector cell. The in vitro sensitized cells were distinctly different morphologically. They were larger and very granular compared with the anti-CD3/IL-2 activated cells. More-

TABLE 3. *Optimal conditions of anti-CD3/IL-2 tumor-draining lymph node cell generation*

Experiment 1			Experiment 2		
Anti-CD3 concentration (μg/ml)[a]	Expansion[b]	Mean no. pulmonary metastases (SEM)[c]	IL-2 concentration (U/ml)[d]	Expansion[b]	Mean no. pulmonary metastases (SEM)[c]
—	—	243 (7)	—	—	227 (13)
100	×7.3	226 (16)	1,000	×12.3	140 (33)
10	×6.3	232 (15)	100	×8.8	184 (26)
1	×5.8	22 (7)	10	×5.5	1 (1)
0.1	×2.7	155 (27)	2	×1.7	3 (2)
0.01	×1.2	218 (19)	0	×0.6	231 (18)
0	×0.7	>250			

Modified from Yoshizawa et al. (56).

[a] TDLN cells bearing 13-day MCA 106 tumors were activated by indicated concentrations of anti-CD3 monoclonal antibody for 2 days, followed by expansion in 10 U/ml IL-2 for 3 days.

[b] Overall expansion after 5 days of culture.

[c] B6 mice were injected i.v. with 106 MCA 10^6 tumor cells to initiate pulmonary metastases. On day 3, activated LN cells ($6–12 \times 10^6$) were given i.v. to each mouse. IL-2 (15,000 U) was given i.p. twice a day for 4 days. Lungs were harvested, and metastases were counted 21–24 days after tumor inoculation.

[d] TDLN cells bearing 13 day MCA 106 tumors were activated with 1 μg/ml anti-CD3 monoclonal antibody for 2 days followed by expansion in the indicated concentrations of IL-2 for 3 days.

over, the CD4/CD8 cell ratio generated by each technique was different. Cells cultured by IVS were >80% $CD8^+$ cells, whereas anti-CD3/IL-2 activated cells were only 60% $CD8^+$. The $CD4^+$ cells were required for maturation of the $CD8^+$ cells during anti-CD3 activation, while $CD4^+$ cells in in vitro sensitized activation could be replaced by IL-2 (57). After activation by either method, it was the $CD8^+$ T-cell subpopulation that mediated regression of pulmonary metastases after adoptive transfer, with exogenous IL-2 helping to maintain their survival and function after transfer.

This anti-CD3/IL-2 activation process generated effector cells that were effective in the treatment of experimentally induced macrometastatic disease confined to one organ site. In a disseminated metastasis model of B16/BL6 melanoma (Fig. 4), adoptive immunotherapy with anti-CD3/IL-2 activated cells prolonged survival. The poorly immunogenic B16/BL6 murine melanoma is a highly invasive tumor that has the propensity to spontaneously metastasize. Animals inoculated with localized tumor in the footpad die several weeks later of spontaneous visceral metastases in multiple organs after amputation of the primary tumor inoculum. We have documented that adoptive immunotherapy of B16/BL6 tumor-bearing mice with anti-CD3/IL-2 activated cells plus IL-2 significantly prolonged survival and cured mice with spontaneous metastases (58,59).

In addition, anti-CD3/IL-2 activated cells can be used to eradicate intracranial tumors despite the fact that the brain is a potentially immunoprivileged site. In experimental studies we performed, animals were transcranially inoculated with 10^5 MCA 205 tumor cells. Without treatment, mice succumbed within 2–3 weeks; however, after systemic adoptive transfer of anti-CD3/IL-2 activated cells, the majority of mice were permanently cured and resisted subsequent intracranial or intravenous tumor challenges (J. Sussman, unpublished observations). As with the treatment of pulmonary metastases, this effect was dose related and immunologically specific. However, in contrast to their effect in the treatment of pulmonary metastases, the adoptively transferred cells were less effective in the presence of exogenously administered IL-2. We are now conducting human trials to evaluate the efficacy of anti-CD3/IL-2 activated vaccine-primed lymph node cells in the therapy of metastatic melanoma and renal cell carcinoma.

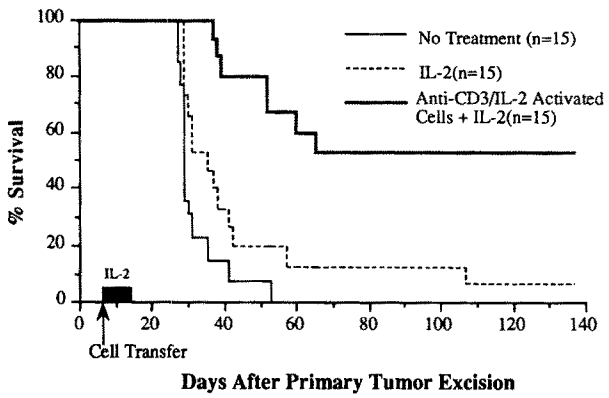

FIG. 4. Therapeutic efficacy of anti-CD3/IL-2 activated cells plus IL-2 in the therapy of spontaneous B16-BL6 melanoma metastases. Mice were inoculated with 10^6 tumor in the footpad and underwent amputation of the primary tumor ~3 weeks later, when spontaneous visceral metastases were established. Seven days after amputation, groups of mice were given no treatment, IL-2 only (15,000 U i.p. twice daily × 7 days), or 10^8 anti-CD3/IL-2 activated cells plus IL-2 (58).

The use of anti-CD3 monoclonal antibodies is only one method to activate T cells in vitro that obviates the need for large amounts of autologous tumor for stimulation. Tuttle et al. have used the protein kinase activator bryostatin 1 and a calcium channel ionophore, ionomycin, to activate TDLN cells. They were able to show specific antitumor effects mediated by these activated cells (60). More recently, in our laboratory, we have exploited the T-cell activating properties of the bacterial enterotoxins known as superantigens. These compounds activate large proportions of T cells based on their usage of particular T cell receptor variable chains. Using three toxins (SEA, SEB, and SPE A) to stimulate preeffector cells from MCA 205 TDLNs in vitro, we have found that T cells bearing Vβ8 may preferentially respond to progressively growing tumors than T cells expressing Vβ3 and Vβ11 (61). These observations may allow us to pinpoint more selective strategies to stimulate tumor-immune T cells for the generation of effector cells.

Hampering the development of effective immunotherapy of human cancer is the poorly defined immunosuppression that occurs in cancer patients. North and co-workers verified the presence of tumor suppressor cells in tumor-bearing hosts that abrogated the antitumor reactivity of adoptively transferred immune lymphocytes (23). This suppression was eliminated by treating the tumor-bearing host with whole-body irradiation or administering cyclophosphamide before transfer of immune cells. There is significantly less information regarding the phenomenon of tumor-induced suppression that

TABLE 4. *Immunologically specific suppression of the host immune response to subcutaneous tumor*

Source of TDLNs[a]		Adoptive immunotherapy[b]	
Subcutaneous tumor	Visceral tumor	Cells transferred	Mean no. pulmonary metastases (SEM)
—	—	—	>250
MCA 106	None	+	1 (<1)
MCA 106	MCA 106	+	240 (10)
MCA 106	MCA 205	+	1 (<1)

[a] For the generation of effector cells, mice were inoculated s.c. in the flank with MCA 106 tumor. Some mice were also inoculated the same day i.v. with the indicated tumor to establish a visceral tumor burden. Twelve days later, the subcutaneous tumor-draining LNs were activated by the anti-CD3/IL-2 method.

[b] Mice with established 4-day MCA 106 pulmonary metastases were given 2×10^7 anti-CD3/IL-2 activated cells. All mice received IL-2 (15,000 U twice daily) for 4 days after transfer of cells.

may inhibit the development of immune cells. Until recently, experimental models of adoptive immunotherapy (i.e., TILs, IVS, or anti-CD3/IL-2 stimulated lymph nodes) have used lymphoid cells derived from donor animals bearing localized subcutaneous tumors in the absence of visceral tumors. We have found that the presence of visceral tumor can suppress the development of sensitized lymphocytes obtained from lymph nodes draining subcutaneous tumors in the same host (Table 4) (V. Sondak, unpublished observations). Thus, the ability to isolate antitumor effector cells from the tumor-bearing host remains an important area of experimental investigation.

CONCLUSION

Advances in the immunologic treatment of tumors have paralleled our understanding of the mechanisms that play a part in T-cell antigen recognition. As we learn more about T-cell-receptor structure, signal transduction pathways, and the structure of antigens, alternate methods to activate T cells reactive to tumor antigens should become apparent. Advances in molecular genetic techniques may be used in the future to modulate the immune system in favor of mediating regression of established malignancies. Our laboratory as well as others have shown that the immunobiology of tumors can be altered by genetically modifying tumor cells to express allogeneic MHC class I antigens (11); costimulatory molecules, such as the B7 ligand (12,13); or suppressive growth factors, such as IGF-I (62). They may also be genetically engineered to secrete cytokines (6–10). The significance of these findings in the development of clinical immunotherapeutic approaches remains to be established. It is also possible that genes may be directly targeted into antitumor T cells, either by in vivo or in vitro techniques, to enhance their survival or therapeutic activity. To date, it is clear that T-cell therapy of human cancer is feasible and therapeutically effective in selected patients. Methods to improve our ability to develop tumor-specific immune T cells will greatly improve the application of this approach to a broader range of patients.

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