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ORIGINAL ARTICLE

Adoptive Cellular Therapy of Malignancy

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The realization that human cancers can be responsive to the manipulation of the immune system has only recently been documented. The immune approaches to the treatment of malignancy can be broadly classified into either active or passive immunotherapies. With active immunotherapy, treatment relies on the in vivo stimulation of the endogenous host immune system to react against tumors with the administration of biological response-modifying agents (ie, bacterial adjuvants, cytokines, tumor vaccines). With passive immunotherapy, treatment involves the delivery of biologic reagents with established tumor-immune reactivity (ie, antibodies or cells) that can directly or indirectly mediate antitumor effects and does not necessarily depend on an intact host immune system. Cellular therapy of malignancy has become more feasible with increased understanding of the interactions between immune cells and tumors. This article will review our current understanding of the principles underlying these interactions.

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HISTORICAL PERSPECTIVE

The feasibility of adoptive immunotherapy of cancer is based on two fundamental observations derived from extensive experimental animal studies. The first of these observations is that tumor cells express unique antigens that can elicit an immune response within the syngeneic host. The other is that the immune rejection of tumors can be mediated by the adoptive transfer of appropriately sensitized lymphoid cells to the tumor-bearing host. Recognizing these fundamental principles required the establishment of animal models consisting of inbred strains of rodents and syngeneic transplantable tumors to eliminate the confounding influences of allograft transplantation immunity observed in earlier studies of tumor rejection in noninbred animals. In 1943, Gross[1] was the first to recognize that inbred mice could be immunized against a tumor that was developed in a mouse of the same inbred strain. This observation was extended by many other investigators with other tumors induced by various agents, and certain basic features were generally noted. Foremost is that individual tumors vary greatly in the strength of their antigenicity, or more appropriately, immunogenicity. The assessment of tumor immunogenicity has traditionally been defined by transplantation procedures in that the immunized host was assessed for rejection of a subsequent challenge of the same tumor. Viral or ultraviolet-induced tumors display the strongest immunogenicity, while chemically induced tumors show intermediate immunogenicity and tumors of spontaneous origin express the poorest immunogenicity. As will be noted later, the immunogenicity of a tumor has a direct influence on the ability to develop immune lymphoid responses against that tumor.

The cellular transfer of immunity was first described by Landsteiner and Chase[2] in 1942, when they reported that delayed

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hypersensitivity to simple compounds could be transferred to naive recipients with cells from peritoneal exudates of sensitized donors. In 1954, Billingham et al[3] described the ability to transfer skin allograft immunity to a normal host with the use of regional lymph node cells from donors that had recently rejected primary skin allografts. They coined the term *adoptive immunity* to describe the acquisition of immunity in a normal subject as a result of the transference, not of preformed antibody, but of immunologically "activated tissue." In 1955, Mitchison[4] reported that the adoptive transfer of lymph node cells from mice that reject tumor allografts would confer accelerated rejection responses in normal animals on being challenged with the specific tumor allograft. More germane to clinical therapy was the ability to transfer immunity to syngeneic tumors. During the ensuing years, several other investigators described animal studies demonstrating the success of rejecting established syngeneic tumors by the systemic transfer of immune "effector" cells.[5,6] In general, animals treated with these approaches have manifested systemic immunity by their ability to reject subsequent tumor challenges in an immunologically specific manner. In these reports, immune effector cells were derived from donor animals subjected to multiple immunization procedures using highly immunogenic tumors. Several important observations were made from these earlier studies. First, large numbers of freshly harvested lymphoid cells from immunized donors were required. At least 10^8 immune cells were needed to mediate tumor regression of palpable subcutaneous tumors in rodents, which meant that several immunized donor animals were necessary to treat one tumor-bearing host. Second, the therapeutic efficacy of these immune cells was directly related to the number of cells transferred. Third, syngeneic immune cells were more effective in mediating tumor regression than were allogeneic or xenogeneic immune cells, which were rapidly eliminated by host immune mechanisms, thus rendering them ineffective. Last, T lymphocytes were found to be responsible for mediating tumor immunity in these adoptive transfer experiments.

OBSTACLES CONFRONTING ADOPTIVE IMMUNOTHERAPY IN HUMANS

As previously indicated, large numbers of immune cells are required to mediate the regression of an established tumor. However, unlike experimental animal systems, humans do not have readily available genetically identical counterparts to obtain immune cells. Therefore, tumor-reactive lymphoid cells will have to be identified and isolated from the patient with cancer. Furthermore, to generate sufficient quantities of immune cells, in vitro methods of expanding these cells while maintaining their immunological reactivities are required to render clinical therapy feasible. These represent formidable obstacles.

Addressing these problems has been hampered by the lack of reliable in vitro assays to predict whether a particular lymphoid cell can mediate tumor regression in vivo. For the most part, the ability of lymphoid cells to lyse tumor cells or proliferate in response to in vitro tumor has no correlation to in vivo therapeutic efficacy. Hence, the ability to identify tumor-reactive immune cells from the tumor-bearing host has been extremely difficult. Moreover, animal studies have demonstrated the existence of tumor-induced immunosuppression that may interfere with the sensitization of lymphoid cells or their ability to mediate antitumor effects.[7-9]

A significant advance in the field was the discovery of interleukin 2, or what was originally called T-cell growth factor.[10] This lymphokine is a 15 000-d glycoprotein elaborated by activated helper T cells and provides the means to culture and expand immune T cells over prolonged periods. In initial animal studies, Cheever et al[11] and Eberlein et al[12] demonstrated that immune cells could be further sensitized in vitro with additional tumor stimulation followed by expansion in the presence of interleukin 2 while still maintaining therapeutic efficacy in adoptive transfer experiments. In addition, several investigators reported enhanced antitumor effects of cultured immune cells in adoptive immunotherapy when concomitant interleukin 2 was administered.[13,14] It was found that the exogenous administration of interleukin 2 induced in vivo proliferation and prolonged survival of the adoptively transferred cells.[15] Hence, interleukin 2 is administered routinely in conjunction with the adoptive transfer of cultured effector cells experimentally and clinically. The basic fundamentals of adoptive immunotherapy established in animal models are summarized in the tabulation below. These preclinical studies paved the way for the initiation of several clinical studies that have demonstrated the feasibility and potential antitumor reactivity of adoptive cellular therapy in humans.

Principles of Adoptive Immunotherapy Established From Animal Models

- The therapeutic efficacy of immune cells depends on the number of cells transferred.
- Tumors vary in degree of immunogenicity.
- There is a lack of in vitro assays that correlate with in vivo antitumor efficacy of immune effector cells.
- Tumor-induced suppression of the host-immune response to tumor has been documented.
- Both fresh or cultured immune lymphoid cells can mediate tumor regression after passive transfer.
- Concomitant administration of interleukin 2 can enhance the in vivo therapeutic activity of cultured immune cells.

NONSPECIFIC LYMPHOKINE-ACTIVATED KILLER CELLS

As described by Grimm et al,[16] early attempts at culturing lymphoid cells in interleukin 2 resulted in the generation of lymphokine-activated killer cells. Lymphokine-activated killer cells were characterized as non-T, non-B lymphoid cells activated in vitro by pharmacologically high concentrations of interleukin 2. These activated cells were found to mediate the nonspecific in vitro cytolysis of autologous as well as allogeneic tumor cells. This phenomenon is in contrast to conventional cytotoxic T lymphocytes in which T cells sensitized to tumor antigens mediate highly specific tumor cytolysis in vitro. The specific cytotoxicity mediated by cytotoxic T lymphocytes requires that they share the same class I major histocompatibility complex antigens as the tumor to which they are sensitized, whereas the nonspecific tumor cytotoxicity mediated by lymphokine-activated killer cells does not have such a restriction. More important, the systemic transfer of lymphokine-activated killer cells was effective in the in vivo treatment of micrometastatic tumor in animal studies.[17,18] Mice with established 3-day pulmonary metastases had significant tumor regression when treated with lymphokine-activated killer cells in conjunction with interleukin 2 (**Figure 1**). These investigations revealed that the in vivo antitumor effects of lymphokine-activated killer cells were nonspecific and highly dependent on the concomitant administration of interleukin 2, but only minimally effective in the treatment of larger macroscopic tumor burdens.

Based on these experimental observations, in 1985, Rosenberg and coworkers[19] at the National Cancer Institute (Bethesda, Md) reported the first human trial of adoptive immunotherapy using autologous lymphokine-activated killer cells and interleukin 2. In that study, 25 patients with advanced cancer (melanoma, renal cell, colon, and lung cancer) were treated with up to 1.8×10^{11} lymphokine-activated killer cells generated from peripheral blood lymphocytes obtained through multiple leukaphoreses, along with bolus infusions of interleukin 2 at maximum tolerated doses. Eleven patients experienced measurable tumor regressions—one complete and 10 partial. This study heralded the feasibility of activating large numbers of ex vivo lymphocytes for adoptive immunotherapy of human cancer. Because this early experience indicated that patients with melanoma and renal cell cancer were responsive, the majority of patients treated in subsequent studies focused on these histologic types of tumor. The **Table** summarizes the results from several institutions of treatment of advanced melanoma and renal cell cancer with lymphokine-activated killer cells and interleukin 2.[20-30] Among 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 patient populations, sites of tumor regression included liver, lung, bone, skin subcutaneous tissue, and lymph nodes. Of note is the observation that if tumor regressed at one site in a patient, it usually was associated with tumor regression at all sites of disease. In addition, a large proportion of patients who experienced complete responses had these responses for a significant duration.

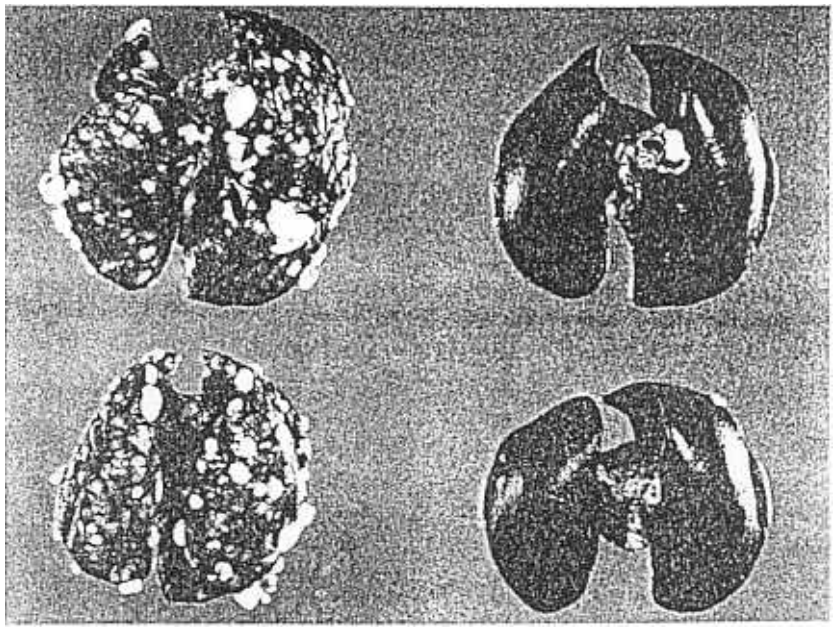

Figure 1. *Adoptive immunotherapy of 3-day MCA-105 pulmonary micrometastases with lymphokine-activated killer cells and interleukin 2. Whole lungs were harvested 14 days after intravenous inoculation with tumor cells and insufflated with india ink via the trachea to allow enumeration of the white lung tumors. Lungs on the left are of control mice that did not receive treatment, and lungs on the right are of animals treated with lymphokine-activated killer cells and nontherapeutic doses of interleukin 2.*

Analyses of factors associated with tumor responses to lymphokine-activated killer cell and interleukin 2 therapy revealed no consistent findings. The number of lymphokine-activated killer cells infused, the in vitro lytic activity of the lymphokine-activated killer cells, or the total amount of interleukin 2 administered did not predict a tumor response. However, these experiences clearly demonstrated that large-scale culture of lymphoid cells for a period of several days could be accomplished without contamination. Intravenous infusions of up to 2×10^{11}

lymphokine-activated killer cells were well tolerated with only fever and chills being the most common side effect. The toxic effects of the therapy were mainly due to interleukin 2 . Multiple organ toxic reactions associated with interleukin 2 can be attributed mostly to the following: (1) induction of a capillary leak syndrome, (2) marked lymphocytic infiltration within visceral organs, and (3) elaboration of other cytokines in response to interleukin 2.[31] The severity of these toxic effects were clearly associated with the cumulative amount of interleukin 2 administered. Fortunately, these toxic effects were quickly reversible once interleukin 2 therapy was discontinued.

Along with the initial trials of lymphokine-activated killer cell therapy, concurrent studies documented the antitumor efficacy of interleukin 2 alone. Using the maximum tolerated dose of interleukin 2, the National Cancer Institute was the first to report tumor responses with active interleukin 2 therapy in patients with melanoma and renal cell cancer.[32] The response rates in these tumors appeared to be comparable to what was achieved with lymphokine-activated killer cells and interleukin 2. The mechanisms postulated for the antitumor activity include (1) the in vivo induction of lymphokine-activated killer cells, (2) the in vivo induction of tumor-sensitized cytotoxic T lymphocytes, or (3) the elaboration of other cytokines (ie, tumor necrosis factor α, interferon-γ, interleukin 6, etc). Because of these findings, it was uncertain whether the combined treatment with lymphokine-activated killer cells and interleukin 2 was significantly better than treatment with interleukin 2 alone. In a multiinstitutional trial, 167 patients with advanced melanoma and renal cell cancer were randomized to receive treatment with either lymphokine-activated killer cells and interleukin 2 or only interleukin 2.[33] In patients with melanoma, the response rates for treatment with lymphokine-activated killer cells/interleukin 2 and only interleukin 2 were 12% and 16%, respectively. In patients with renal cell cancer, the response rates for treatment with lymphokine-activated killer cell/interleukin 2 and interleukin 2 only were 13% and 8%, respectively. These results indicate that the addition of lymphokine-activated killer cells did not improve response rates. A separate randomized trial of lymphokine-activated killer cells/interleukin 2 vs interleukin 2 alone in 181 patients with advanced cancer was recently reported by Rosenberg et al from the National Cancer Institute.[34] Of the 181 patients, 97 had renal cell cancer and 54 had melanoma. There were 10 complete responses among the 85 assessable patients who received lymphokine-activated killer cells/interleukin 2, compared with four among the 79 who received interleukin 2 alone. There were 14 reponses and 12 partial responses. There was a trend toward increased survival when lymphokine-activated killer cells/interleukin 2 was administered in patients with melanoma, but no trend was observed for patients with renal cell cancer. Because of the limited contribution of lymphokine-activated killer cells compared with interleukin 2 therapy alone, the use of tumor-sensitized T cells appears to hold more promise for successful adoptive immunotherapy.

TUMOR-SENSITIZED T LYMPHOCYTES

Tumor-Infiltrating Lymphocytes

In contrast to lymphokine-activated killer cells, which can be readily generated from peripheral blood lym-

Tumor Response to Treatment of Advanced Melanoma and Renal Cell Cancer With Lymphokine-Activated Killer Cells and Interleukin 2*

Study, y	Lymphokine-Activated Killer Cells†	Melanoma				Renal Cell Cancer			
		Total No.	CR	PR	CR and PR	Total No.	CR	PR	CR and PR
Rosenberg,[20] 1988	7.9×10^{10}	34	3	3	6	54	7	10	17
West et al,[21] 1987	$6.8\text{-}9.1\times10^{10}$	10	0	5	5	6	0	3	3
Schoof et al,[22] 1988	4.3×10^{10}	9	1	4	4	10	0	5	5
Thompson et al,[23] 1989	$3.4\text{-}4.3\times10^{10}$	8	0	0	0	8	1	0	1
Paciucci et al,[24] 1989	3.4×10^{10}	5	0	1	1	9	0	1	1
Dutcher et al,[25] 1989	8.9×10^{10}	36	1	5	6	...	...	...	...
Bar et al,[26] 1990	8.3×10^{10}	55	1	6	7	...	...	...	...
Dutcher et al,[27] 1991	1.6×10^{11}	33	0	1	1	...	...	...	...
Wang et al,[28] 1989	$4.9\text{-}6.1\times10^{10}$	...	...	...	...	32	2	5	7
Fisher et al,[29] 1988	7.0×10^{10}	...	...	...	...	32	2	3	5
Parkinson et al,[30] 1990	9.2×10^{11}	...	...	...	...	47	2	2	4
Total	...	**190**	**6**	**25**	**30 (16%)**	**198**	**14**	**29**	**43 (22%)**

**CR indicates complete response or regression of all evaluable tumors; PR, partial response or greater than or equal to 50% regression of all evaluable tumors.*

†Mean or median number of cells.

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(**Figure 2**). Heretofore, lymphokine-activated killer cells and interleukin 2 were unable to successfully cure advanced tumors in these murine models. In the murine tumor-infiltrating lymphocyte studies, the use of cyclophosphamide appeared to be necessary before cell transfer and interleukin 2 administration role of certain

ation of tumor-induced suppres-
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Th .ro cyt

infiltrating lymphocytes clearly distinguished their immunologic function from lymphokine-activated killer cells. Lymphokine-activated killer cells were nonspecifically reactive to a broad spectrum of tumors in the 4-hour chromium 54–release cytotoxicity assay. In contrast, tumor-infiltrating lymphocytes have been demonstrated to display tumor-specific reactivity to immunologically distinct murine tumors.[36] In human studies, approximately 30% of tumor-infiltrating lymphocytes from patients with melanoma have been reported to exhibit highly specific cytolytic reactivity against autologous tumor and not to other allogeneic tumors.[37] These findings suggested that there are distinct tumor-specific antigens among melanoma tumors to which T cells can be immunized.

Based on these reports, the National Cancer Institute undertook clinical studies of tumor-infiltrating lymphocytes. In these studies cyclophosphamide was administered 36 hours before tumor-infiltrating lymphocyte infusion and interleukin 2 administration based on the effectiveness of this combination therapy in the animal studies. In an earlier study, the National Cancer Institute reported that 11 of 20 patients with advanced melanoma responded to this approach.[38] In a subsequent report that extended this experience to 55 patients, 22 patients (40%) with melanoma experienced significant tumor regression with tumor-infiltrating lymphocyte and interleukin 2 therapy, which included a subgroup of patients who did not receive cyclophosphamide.[39] In this trial, lysis of autologous tumor cells by tumor-infiltrating lymphocytes in an in vitro assay was associated with the development c :esponse
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tients with melanoma and renal cell cancer. Bukowski et al[41] reported no responses in 18 patients with renal cell carcinoma who were treated with large numbers of tumor-infiltrating lymphocytes and increasing doses of interleukin 2. All of these studies have demonstrated extremely diverse immune characteristics among individual tumor-infiltrating lymphocytes cultures. Future studies will focus on culture methods or selection procedures to grow the relevant subpopulations of tumor-infiltrating lymphocytes that mediate tumor-specific reactivity.[42]

Tumor-Sensitized Lymph Node Cells

An alternative source of effector cells, in addition to the tumor, is the draining lymph nodes. Forty years ago, it was shown that draining lymph node cells from donors who reject primary allografts could confer skin or tumor allograft immunity to naive recipients.[3,4] More recently, our laboratory demonstrated that the induction of systemic immunity by vaccination of the host with autologous tumor cells and a bacterial adjuvant can be abrogated by the early removal of the lymph nodes draining the vaccine site.[43] In the last several years, we observed tumor-draining lymph nodes to harbor lymphoid cells that are not functionally capable of mediating tumor rejection in adoptive transfer experiments; however, further in vitro

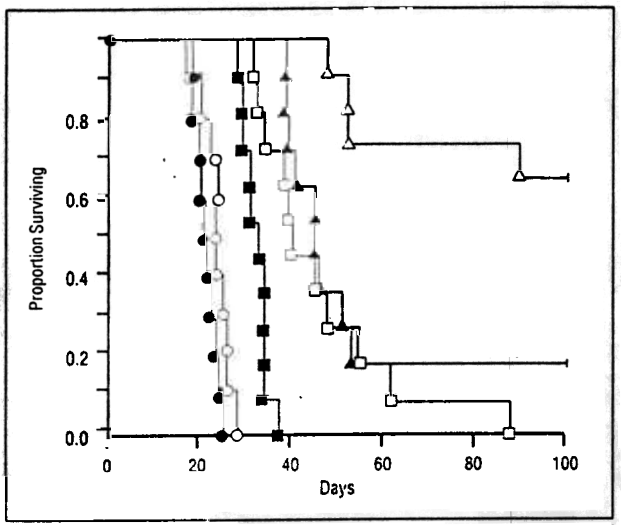

Figure 2. *Treatment of mice with advanced pulmonary metastases from the immunogenic MC-38 colon adenocarcinoma. This figure summarizes two experiments in which treatment was begun 12 and 14 days, respectively, after intravenous injection of tumor cells. Mice received 100 mg/kg of intravenous cyclophosphamide 6 hours before receiving intravenous tumor-infiltrating lymphocytes (2×10^7 to 2.4×10^7 cells) and interleukin 2 (20 000 U intraperitoneally every 8 hours for 5 days). Substantial improvement in survival was seen with cyclophosphamide, interleukin 2, and tumor-infiltrating lymphocyte therapy.[35] Closed circles indicate no treatment; open circles, interleukin 2; closed squares, cyclophosphamide; open squares, cyclophosphamide and interleukin 2; closed triangles, cyclophosphamide and tumor-infiltrating lymphocytes; and open triangles, cyclophosphamide, interleukin 2, and tumor-infiltrating lymphocytes.*

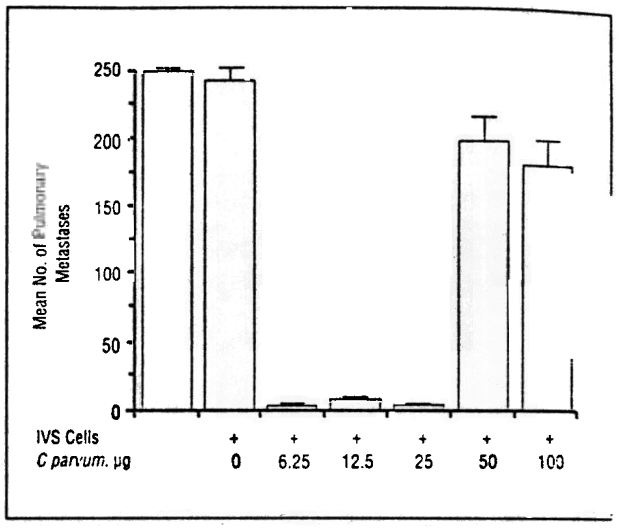

Figure 3. *Treatment of poorly immunogenic MCA-102 pulmonary micrometastases with tumor-sensitized lymph node cells activated by in vitro sensitization (IVS) culture. Mice were inoculated subcutaneously with tumor cells admixed with various amounts of* Corynebacterium parvum. *Draining lymph node cells were removed 7 days later, cultured with IVS, subsequently harvested, and adoptively transferred (1.5×10^7 cells intravenously) to mice with 3-day pulmonary metastases along with the administration of interleukin 2 (7500 U intraperitoneally twice daily for 4 days). Mouse lungs were harvested and metastatic nodules counted 14 days after tumor inoculation.* Corynebacterium parvum *was found to be a significant adjuvant in eliciting tumor-sensitized lymph node cells and was dose dependent.[45]*

activation of these cells resulted in the generation of tumor-specific T cells that were therapeutically effective. One approach to the activation of these lymph node cells involves their in vitro sensitization (IVS) with irradiated tumor cells in the presence of low concentrations of interleukin 2.[44,45] During IVS culture, lymphoid cells differentiate and numerically expand into potent effector cells that can mediate the regression of advanced, macroscopic tumors established in visceral organs.[46] Of significance was the ability to generate effective IVS cells against a defined poorly immunogenic tumor. It has been postulated that human tumors are poorly immunogenic based on their spontaneous origin and that many animal tumors are not relevant for study because they are significantly immunogenic.[47] We, therefore, examined the ability to generate immune T cells reactive to the MCA-102 sarcoma, a defined poorly immunogenic tumor. Animals cannot develop systemic immunity to these tumors with several conventional vaccination procedures, and therapeutic tumor-infiltrating lymphocytes cannot be generated with standard techniques from these tumors.[36,48] In addition, tumor-draining lymph node cells could not be successfully stimulated with IVS culture to develop into immune T cells. However, we discovered that the subcutaneous inoculation of MCA-102 tumor cells admixed with the bacterial adjuvant, *Corynebacterium parvum*, resulted in reactive draining lymph node cells that could be subsequently cultured with the IVS method to generate therapeutic T-effector cells (**Figure 3**).[49] Moreover, only lymph nodes draining this tumor "vaccination" site were capable of differentiating into immune T cells during IVS culture; peripheral blood lymphocytes, splenocytes, distant lymph nodes and bone marrow cells were not effective.

Based on these experimental observations, we investigated the immune function of tumor-sensitized lymph node cells in the treatment of patients with advanced cancer.[50] Patients with melanoma or renal cell cancer received intradermal inoculations of autologous tumor cells admixed with BCG vaccine, which was chosen because of its documented efficacy as an immune adjuvant in clinical studies using autologous tumor vaccinations.[51,52] Lymph nodes draining the vaccine sites were harvested 10 days later and cultured with the IVS method. A mean of 7×10^9 IVS cells was infused into 10 patients in conjunction with low-dose interleukin 2. Delayed hypersensitivity to their autologous tumor was seen in the majority of patients (78%) infused with IVS cells compared with none from a group of patients who received tumor vaccination and interleukin 2 without the transfer of activated cells. This suggested that immune reactivity against tumor was mediated by the transferred cells. Tumor regression was observed in one patient; however, the limited number of cells infused did not permit an adequate assessment of the antitumor effects of these cells. Since large quantities of tumor cells were required for IVS

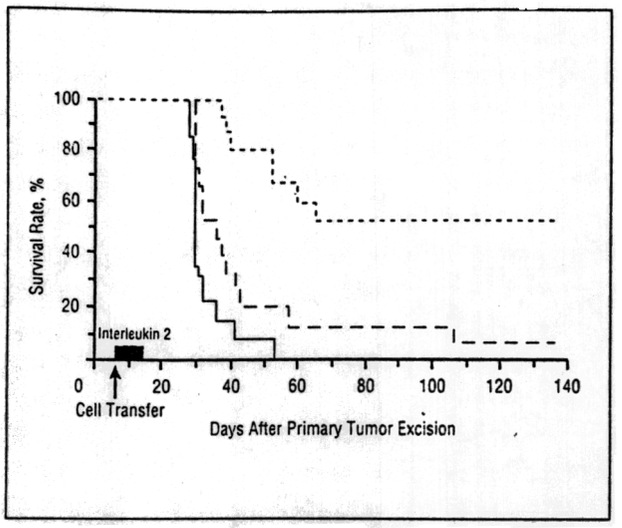

Figure 4. *Therapeutic efficacy of anti-CD3/interleukin 2–activated cells and interleukin 2 in the therapy of spontaneous B16-BL6 melanoma metastases. Mice were inoculated with 10^6 tumor cells in the footpad and underwent amputation of the primary tumor approximately 3 weeks later when spontaneous visceral metastases were established. Seven days after amputation, groups of mice were given no treatment (n=15; solid line), interleukin 2 only (15 000 U intraperitoneally twice daily for 7 days; n=15; dashed line), or anti-CD3/interleukin 2–activated cells (10^6) and interleukin 2 (n=15; dotted line).*[56]

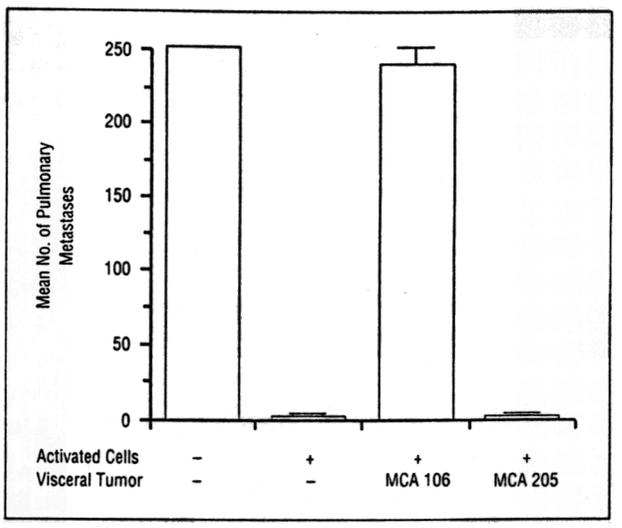

Figure 5. *Immunologically specific suppression of the host-immune response to subcutaneous tumor. Mice were inoculated intravenously with MCA 106 or MCA 205 to establish lung metastases. At the same time, all animals were inoculated subcutaneously in the flank with MCA-106 tumor cells. Lymph node cells draining the subcutaneous MCA-106 tumor cells were retrieved for anti-CD3/interleukin 2 activation and assessed for antitumor efficacy in the treatment of 4-day MCA-106 pulmonary metastases. Lymph node cells derived from animals without concomitant lung tumor or with the unrelated MCA-205 tumor were significantly more effective than the cells from animals with MCA-106 lung tumor, which suppressed the immune response elicited by the flank tumor.*

culture, repeated activation cultures were not feasible to expand cells further.

Alternate in vitro methods are needed to activate these tumor-sensitized lymph node cells without the limiting requirement of tumor cells that occurs in the clinical setting. In this regard, we recently found that antibodies that bind to the T-cell receptor/CD3 complex (anti-CD3) can mimic antigen in the stimulation of tumor-sensitized T cells.[53,54] Even though anti-CD3 can nonspecifically activate resting T cells, the sequential culture of tumor-draining lymph node cells with anti-CD3 and low-concentration interleukin 2 resulted in an expanded population of antitumor-reactive T cells with exquisite immunologic specificity. Application of this anti-CD3/interleukin 2 activation procedure was examined with the poorly immunogenic B16-BL6 murine melanoma, which is a highly invasive tumor of spontaneous origin. Because of its weak immunogenicity, anti-CD3/interleukin 2 activation of lymph node cells draining this tumor did not result in the generation of therapeutically effective cells. However, we found that therapeutic anti-CD3/interleukin 2–activated cells could be reliably generated from lymph nodes draining inoculation sites of B16-BL6 tumor admixed with *C parvum*.[55] In addition to the ability to mediate regression of experimentally induced pulmonary metastases, these activated cells were effective in the treatment of spontaneous visceral metastases originating from a primary tumor, a condition that is relevant to clinical therapy (**Figure 4**).[56] Tuttle and colleagues[57] further documented the ability to obtain immune T cells from tumor-draining lymph nodes using another method to activate these cells in vitro for subsequent adoptive immunotherapy. Using bryostatin 1, a novel protein kinase activator, and ionomycin, a calcium ionophore, they were able to generate therapeutically effective tumor-specific T cells from tumor-draining lymph nodes. Since the anti-CD3/interleukin 2 and bryostatin-ionomycin activation methods do not require tumor antigen, these techniques may prove useful for the generation of activated T cells in sufficient numbers for meaningful clinical therapy. We are currently evaluating the therapeutic efficacy of anti-CD3/interleukin 2 activated cells in a clinical study.

FUTURE DIRECTIONS

The adoptive transfer of tumor-reactive lymphoid cells as therapy for malignant neoplasms represents an attractive alternative to conventional treatment modalities. Among different effector cells, T lymphocytes with specific immune reactivity to the tumor are more potent than nonspecific lymphokine-activated killer cells. Hence, the direction of this field has focused on the development of methods to isolate and expand tumor-specific T lymphocytes. Although considerable progress is being made in both animal and human studies, this approach to cancer treatment is still in its infancy and a variety of problems remain unresolved. The following tabulation

summarizes the factors that should be considered in devising clinical adoptive immunotherapeutic strategies in humans.

Requirements for Successful Adoptive Immunotherapy in Humans

- Isolation of tumor-reactive lymphoid cells
- In vitro methods to generate large numbers of tumor-reactive cells for adoptive transfer
- Possible down-regulation of tumor-induced immunosuppression in the patient

The ability to retrieve tumor-sensitized cells from the total pool of lymphoid cells available in the patient is of foremost importance. Human cancers spontaneously arise and may not be sufficiently immunogenic to allow the isolation of immune T cells. Several approaches have been reported to convert poorly immunogenic tumors into immunogenic ones, such as xenogenization with chemical mutagens or viruses,[58,59] and more recently, genetically modifying tumors to elaborate cytokines or express foreign antigens.[60,61] Several reports demonstrated that the transfection or transduction of cytokine genes (ie, tumor necrosis factor α, interferon γ, interleukin 2 , interleukin 4, granulocyte-macrophage colony-stimulating factor) into murine tumor cells will prevent the growth of an inoculum of the modified tumor cells in a normal host.[62-67] This rejection response appears to be related to the elaboration of the cytokine in the microenvironment of the tumor cells, which results in the subsequent recruitment of endogenous host-immune cells. Although local tumor rejection is observed, the regression of established unmodified tumors in separate sites has not been observed to date. Hence, the use of genetically modified tumors as therapeutic vaccines may have limited applications. We have begun to examine the use of genetically modified tumors to generate immune T cells for adoptive immunotherapy. In preliminary studies, we used a novel in vivo gene transfer technique to transfect the poorly immunogenic B16-BL6 melanoma with a gene encoding an allogeneic class I major histocompatibility antigen.[68] This elicited sensitized T cells in the draining lymph nodes, which were effective in adoptive immunotherapy experiments after ex vivo anti-CD3/interleukin 2 activation. The difficulties associated with the identification of suitably altered tumor cells as well as defining their immunogenicity will require a fundamental understanding of the involved mechanisms before these approaches can be applied successfully for inducing specific T-cell responses against human tumors.

Another possible obstacle in the retrieval of sensitized lymphoid cells from the tumor-bearing host is the phenomenon of tumor-induced immunosuppression. Nonspecific immunosuppression engendered by the tumor-bearing state has been described in animals and humans, and is too broad a subject to review here. More germane to T-cell therapy is the phenomenon of specific tumor-induced suppression, which is postulated to be mediated by tumor-suppressor cells. North[7] elegantly demonstrated the presence of tumor-suppressor cells in tumor-bearing hosts, which abrogated the antitumor reactivity of adoptively transferred immune lymphocytes. This suppression was eliminated by treating the tumor-bearing host with whole-body irradiation or administering cyclophosphamide before the transfer of immune cells. There is significantly less information on tumor-induced suppression that may inhibit immune cell development. Until recently, experimental models of adoptive immunotherapy (ie, tumor-infiltrating lymphocytes or tumor-sensitized lymph nodes) have used lymphoid cells derived from donor animals bearing localized subcutaneous tumors in the absence of concomitant visceral tumors. We 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 (**Figure 5**).[9] This highlights the complexity of the tumor-bearing state that can negatively modulate immune responses to tumor antigens. The ability to isolate antitumor effector cells from the tumor-bearing host remains an important area of experimental investigation.

Successful adoptive immunotherapy requires the availability of large numbers of appropriately reactive effector cells. This requirement underscores the need for developing methods to facilitate the long-term expansion of effector T lymphocytes while maintaining their specificity and ability to function in vivo. The most productive approach for long-term growth of sensitized T cells for cancer therapy is not yet defined, although many techniques are being investigated. The conventional mixed lymphocyte-tumor interactions might yield cells with specific antitumor reactivity, but are poorly suitable for significant proliferation of T cells. The addition of interleukin 2 to mixed lymphocyte-tumor cultures has been shown to enhance sensitization to tumor antigens and T-cell expansion.[45,69] Indeed, the methods for generating tumor-infiltrating lymphocytes and IVS cells are analogous to the culture of T cells with both antigenic and interleukin 2 stimulations. However, the optimal conditions for IVS as well as expanding tumor-specific T cells have not been established. It is possible that through the use of additional cytokines or T-cell antibodies, culture systems may be designed to selectively stimulate the growth of particular T-cell subpopulations that are more suitable for adoptive immunotherapy.

Adaptation of modern techniques of molecular biology may also prove invaluable in designing

strategies to generate potent antitumor effector cells. Recent reports on the feasibility and safety with which tumor-infiltrating lymphocytes transduced with the neomycin-resistant gene were successfully introduced into humans has generated overwhelming interest in the application of gene-transfer technology to T-cell therapy of human malignancy.[70] As proposed by Rosenberg et al,[70] tumor-infiltrating lymphocytes could be transduced with vectors expressing tumoricidal cytokines such as tumor necrosis factor, which might then improve their antitumor efficacy. The underlying rationale for this approach is that the in vivo antitumor efficacy of sensitized T lymphocytes is governed not only by specific tumor recognition through the T-cell receptor but also by the necessary machinery to produce certain cytokines.[41] While this hypothesis is attractive and has some scientific validity, much more work needs to be done to provide direct evidence as to which cytokines are important.

This review summarizes our current understanding on the cellular therapy of cancer. The experimental observations that appropriately activated lymphoid cells can mediate regression of established tumor has led to the institution of clinical trials with encouraging results. Despite this limited success, further elucidation of the principles involved in sensitizing T cells to tumor antigens will allow broader applications of this therapeutic modality.

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