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Distinct Immunologic Specificity of Tumor Regression Mediated by Effector Cells Isolated from Immunized and Tumor-Bearing Mice[1]

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Sensitized T lymphocytes can mediate potent antitumor effects when transferred to tumor-bearing animals. Employing the MCA 105 and MCA 106 sarcomas, we were able to generate antitumor effector cells by immunization of syngeneic mice with tumor cells admixed with *Corynebacterium parvum*. These immune splenocytes could be further sensitized and expanded in culture by the *in vitro* sensitization (IVS) method utilizing tumor stimulator cells and IL-2. Adoptive immunotherapy of pulmonary metastases mediated by noncultured splenocytes from immunized mice or immune IVS cells showed exquisite specificity between the two sarcomas. These results demonstrate the presence of tumor-specific antigens on MCA 105 and MCA 106 tumor cells which can serve as target molecules for immunotherapy. Recently, we have generated therapeutic T lymphocytes from mice bearing progressively growing tumors by the IVS method. However, IVS cells from tumor-bearing mice showed cross-reactivity between the MCA 105 and 106 sarcomas in adoptive immunotherapy experiments. Since these IVS cells did not affect other control tumors, the limited cross-reactivity suggests the presence of common tumor-associated antigens on MCA 105 and MCA 106 tumor cells which can also serve as the target for tumor rejection. Therefore, immune responses to progressive tumor growth and to immunization are distinct with respect to antigen recognition by T lymphocytes.

INTRODUCTION

Adoptive immunotherapy of tumors with specifically sensitized T lymphocytes has been demonstrated to be a curative therapeutic manipulation in a variety of animal models (1–7). Since successful therapy requires large numbers of syngeneic antitumor lymphocytes, such an approach in the clinical setting will likely require the ability of obtaining antitumor reactive lymphoid cells from the patient and subsequent expansion *in vitro* to large enough numbers for therapeutic purposes. In order to define conditions necessary for successful adoptive immunotherapy, we have developed murine tumor models involving methylcholanthrene-induced sarcomas known as MCA 105 and MCA 106 (6). In contrast to the highly immunogenic FBL-3 lymphoma and Meth A tumors which have commonly been used in previous adoptive

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immunotherapy studies, these tumors are weakly immunogenic to their syngeneic hosts. Hence, these tumors provide more realistic animal models for mechanistic studies of adoptive immunotherapy. In addition, these two tumors were similarly induced and afforded excellent specificity controls for experimental studies.

In animal studies, one of the sources of therapeutic T lymphocytes can be obtained from animals that have been immunized to resist tumor challenge. *In vivo* immunization to MCA 105 and MCA 106 can be performed by inoculating mice with viable tumor cells admixed with *Corynebacterium parvum.* Spleen cells from immune animals will cause the regression of established local and disseminated tumor when transferred (6). Using one of these models, MCA 105, we developed an *in vitro* sensitization (IVS) method to secondarily stimulate immune lymphoid cells with irradiated tumor cells in the presence of IL-2 (8). In addition to numerical expansion, we found that these immune IVS cells had increased *in vivo* antitumor reactivity compared to noncultured fresh immune cells. Phenotype analysis of immune effector T cells revealed that the IVS procedure induced differentiation of therapeutic T lymphocytes (9).

Recently, we have reported that the IVS method can be used to generate therapeutic effector cells from animals bearing progressively growing MCA 105 tumors (10). Lymphoid cells freshly isolated from the tumor-bearing host do not cause tumor regression after adoptive transfer. However, after IVS, these "preeffector" lymphoid cells differentiate into effector cells capable of mediating potent antitumor effects (10). The presence of preeffector cells in the tumor-bearing host was most consistantly observed in the lymph nodes draining a progressively growing tumor.

In this report, we have extended these observations to an additional syngeneic tumor model, MCA 106. Because of the availability of two similar murine sarcomas, we examined the immunologic specificity of tumor regression after therapy with effector cells obtained from immunized and tumor-bearing mice. Our results demonstrate that adoptive transfer of effector cells from immunized animals before or after IVS resulted in immunologically specific antitumor reactivity. These findings clearly indicate the existence of tumor-specific rejection antigens on each tumor. However, effector cells generated from tumor-bearing mice demonstrated cross-reactivity between the MCA 105 and MCA 106 tumors. Therefore, in addition to individual tumor-specific rejection antigens, there exists common tumor-associated antigens that can also serve as targets for tumor rejection. More importantly, our data indicate that the immune responses elicited by immunization and during progressive tumor growth are distinct with respect to antigen recognition.

MATERIALS AND METHODS

Mice. Female C57BL/6J (B6) mice, 8 to 12 weeks old, were obtained from the Jackson Laboratory, (Bar Harbor, ME). Caged in groups of six or fewer, the animals were fed NIH laboratory chow and were given water *ad libitum.*

Tumor. The MCA 105 and MCA 106 sarcomas are methylcholanthrene-induced tumors of B6 origin and are antigenically distinct by tumor immunization and tumor challenge experiments (6). A large number of vials of these tumors from the first passage generation were cryopreserved. After thawing from storage, these tumor were transplanted by subcutaneous inoculation in syngeneic mice and were always used

within the first six transplant generations. Other similarly induced MCA 101 and MCA 102 sarcomas of B6 origin served as additional specificity controls. Single cell suspensions were prepared from solid tumors by digestion with constant stirring in 40 ml of Hanks' balanced salt solution (HBSS) (Biofluids, Rockville, MD) containing 4 mg deoxyribonuclease, 40 mg collagenase, and 100 U hyaluronidase (Sigma Chemical Co., St. Louis, MO) for 3 hr at room temperature (6).

For immunization to the MCA 105 and MCA 106 tumors, mice were injected either subcutaneously (sc) or into the footpad with a mixture of 1.5×10^6 viable tumor cells and 60 to 100 mcg of formalin-killed *C. parvum* (Burroughs Wellcome Co., Research Triangle Park, NC). Fourteen days after immunization, mice were challenged with 2×10^5 tumor cells intradermally (id). Mice who rejected this tumor challenge were subsequently challenged with 10^6 tumor cells id. This immunization protocol resulted in approximately 50% of the mice developing systemic immunity. Immune splenocytes were obtained from tumor-free mice 2 to 4 weeks after the last tumor challenge.

For establishing solid tumors, B6 mice were inoculated sc in the footpad with 5×10^5 to 10^6 MCA 105 or MCA 106 sarcoma cells in 0.05 ml of HBSS. The growth of tumors was evident on Day 3 and all animals eventually succumbed to the progressively growing tumor with a median survival time of 23 days.

Lymphoid cell suspensions. Spleens from normal or immunized mice as well as lymph nodes (popliteal) draining sites of growing tumor were removed asceptically. Single cell suspensions were prepared mechanically by pressing the blunt end of a 10-ml plastic syringe plunger in HBSS. The cell suspension was filtered through a layer of No. 100 nylon mesh (Nitex: Lawshe Industrial Co., Bethesda, MD), centrifuged, and erythrocytes were lysed by resuspension of the cell pellet in ammonium chloride–potassium lysing buffer (NIH Media Production Section) for 2 min at room temperature. The cells were washed twice and suspended in HBSS for adoptive transfer or in appropriate medium for culture.

Interleukin-2 (IL-2). The recombinant human IL-2, kindly supplied by the Cetus Corporation (Emeryville, CA), had a specific activity of 3×10^6 U/mg protein. The endotoxin level in the purified preparation was <0.1 ng/10^6 U IL-2 as assessed by the standard Limulus assay (11).

IVS of lymphoid cells with tumor cells and IL-2. The procedures for IVS have been detailed previously (10). Briefly, 4×10^5 spleen cells or 2×10^5 lymph node cells were cultured with 2×10^5 MCA 106 or 8×10^5 MCA 105 2000 R irradiated tumor cells in the presence of 1000 U/ml recombinant IL-2 in 2 ml of complete medium (CM) in 24-well plates. The composition of CM has been described elsewhere (10). The cultures were incubated at 37°C, 5% CO_2, and were fed with 1.0 ml of CM containing 1000 U/ml of recombinant IL-2 every 3 days. The cells were routinely harvested on Days 9–11 when they grew to a high density. These cells were washed three times before resuspending in HBSS for adoptive immunotherapy.

Adoptive immunotherapy model. B6 mice were injected iv with 4×10^5 MCA 106, 6×10^5 MCA 105, 8×10^5 MCA 101, or 5×10^5 MCA 102 sarcoma cells in 1.0 ml HBSS to establish pulmonary metastases. In some experiments, mice were given 500 R whole body irradiation prior to tumor inoculation (Gammacell 40; Atomic Energy of Canada, Limited). On Day 3 when multiple foci of pulmonary metastases were microscopically evident, therapy was instituted either with freshly isolated or with

TABLE 1

Specificity of Adoptive Immunotherapy Mediated by Freshly Isolated Immune Splenocytes

Cells transferred[a]	Lung tumor	Mean no. of metastases (SEM) Expt 1	Expt 2
1. —	MCA 105	250	235 (13)
2. Normal	MCA 105	204	232 (13)
3. MCA 105 Immune	MCA 105	2 (1)[b]	17 (7)[b]
4. MCA 106 Immune	MCA 105	161 (29)	250
5. —	MCA 106	250	194 (20)
6. Normal	MCA 106	250	203 (25)
7. MCA 105 Immune	MCA 106	220 (23)	195 (32)
8. MCA 106 Immune	MCA 106	29 (30)[c]	31 (15)[c]

[a] Adoptive transfer of $6–7 \times 10^7$ freshly isolated splenocytes in mice with 3-day established MCA 105 or MCA 106 pulmonary metastases. Pulmonary metastases were established by administering whole body irradiation (500 R) followed by iv inoculation with MCA 105 or MCA 106 tumor cells.

[b] $P < 0.05$ compared to Group 2.

[c] $P < 0.05$ compared to Group 6.

IVS lymphoid cells given in 1.0 ml of HBSS through the tail vein. In experiments where IVS cells were used, mice were also injected intraperitoneally (ip) with recombinant IL-2 (7500 U) in 0.5 ml of HBSS, twice a day for 3 to 4 days starting on the day of cell transfer. In all experiments, at least five mice were included in each treatment group. At Days 14 to 16 after tumor inoculation, the mice were ear-tagged, randomized, and then sacrificed for enumeration of metastatic pulmonary nodules as described (12). Metastases of the sarcoma form white nodules on the blackened surface of the lung (insufflated with a 15% solution of India ink) when "bleached" by Fekette's solution. Nodules were counted in a double-blind fashion without knowledge of the treatment of that mouse. Mice with metastatic foci too numerous to count were assigned an arbitrary value of 250 because this was the largest number of nodules that could be reliably enumerated per lung.

Statistics. The Wilcoxon rank test was used to determine the significance of differences in numbers of pulmonary metastases between treatment groups. Two-sided P values are presented. No mice were excluded from the statistical evaluation.

RESULTS

Specificity of Adoptive Immunotherapy Using Freshly Harvested Immune Splenocytes

In criss-cross experiments, splenocytes from mice immunized to the MCA 105 and MCA 106 tumors were given systemically through the tail vein to hosts bearing 3-day established MCA 105 or MCA 106 pulmonary metastases (Table 1). In two experiments, the adoptive transfer of normal splenocytes had no therapeutic effect. Transfer of MCA 105 immune cells significantly reduced the number of MCA 105 pulmonary metastases but not the MCA 106 tumor. In addition, MCA 106 immune

TABLE 2

Specificity of Adoptive Immunotherapy Mediated by MCA 106 Immune IVS Splenocytes

Cells[a]	Lung tumor	Mean no. of metastases (SEM)		
		Expt 1	Expt 2	Expt 3
1. —	MCA 106	239 (11)	151 (25)	163 (24)
2. $Immune_a$	MCA 106	250	99 (20)	127 (36)
3. Immune	MCA 106	9 (5)[b]	6 (2)[b]	2 (1)[b]
4. —	MCA 105	235 (16)	232 (12)	98 (23)
5. $Immune_a$	MCA 105	250	239 (9)	45 (10)
6. Immune	MCA 105	212 (38)	162 (15)	166 (49)

[a] MCA 106 immune cells were stimulated in IVS with irradiated tumor plus IL-2 or IL-2 alone ($Immune_a$). Adoptive transfer of $1.0–1.2 \times 10^7$ cells was performed in mice with 3-day established MCA 105 or MCA 106 pulmonary metastases. IL-2 was administered for six doses (7500 U) ip twice daily after adoptive transfer. In Experiment 3, mice were given 500 R whole body irradiation prior to tumor inoculation.

[b] $P < 0.01$ compared to Group 2.

cells had a significant antitumor effect against MCA 106 pulmonary metastases and no effect against the MCA 105 tumor. These results demonstrated the immunologic specificity of the adoptive immunotherapy with *in vivo*-immunized effector cells.

Specificity of Adoptive Immunotherapy Using Immune Splenocytes after IVS

Freshly harvested MCA 106 immune splenocytes were restimulated with irradiated tumor in the presence of IL-2 by the IVS procedure. The antitumor efficacy of these IVS cells was examined *in vivo* by adoptive transfer in mice bearing 3-day established pulmonary metastases. In contrast to previous experiments utilizing 6×10^7 fresh immune splenocytes, we have found that $1.0–1.2 \times 10^7$ IVS-expanded cells administered along with low doses of exogenous IL-2 were sufficient to mediate tumor regression. In Table 2, MCA 106 immune IVS cells mediated significant reduction of MCA 106 pulmonary metastases in three experiments. In contrast, MCA 106 immune cells expanded in IL-2, in the absence of tumor stimulation, did not have antitumor efficacy, thus underscoring the necessity of antigenic stimulation. The immunologic specificity of the adoptive immunotherapy mediated by MCA 106 immune IVS cells was also examined by treatment of mice bearing established MCA 105 pulmonary metastases (Table 2). Clearly, MCA 106 immune IVS cells exhibited no therapeutic effect against the MCA 105 tumor.

The specificity of the adoptive immunotherapy mediated by MCA 105 immune IVS cells was examined in similar criss-cross experiments (Table 3). In all three experiments, significant antitumor effects against MCA 105 lung metastases were seen while there was no therapeutic efficacy seen against the MCA 106 tumor in two experiments (Experiment 1 and 2). In Experiment 3, only a modest reduction in MCA 106 tumor was evident compared to MCA 105 tumor. Taken together, these results demonstrate that the MCA 105 and MCA 106 sarcomas possessed individual tumor-specific rejection antigens. Adoptive immunotherapy with spleen cells derived from

TABLE 3

Specificity of Adoptive Immunotherapy Mediated by MCA 105 Immune IVS Splenocytes

Cells[a]	Lung tumor	Mean no. of pulmonary metastases (SEM)		
		Expt 1	Expt 2	Expt 3
1. —	MCA 105	179 (27)	201 (26)	222 (28)
2. $Immune_a$	MCA 105	186 (41)	150 (38)	139 (46)
3. Immune	MCA 105	44 (25)[b]	2 (1)[b]	3 (2)[b]
4. —	MCA 106	164 (24)	222 (21)	250
5. $Immune_a$	MCA 106	126 (9)	216 (30)	250
6. Immune	MCA 106	168 (34)	191 (46)	123 (28)[c]

[a] MCA 105 immune cells were stimulated in IVS with irradiated tumor plus IL-2 or IL-2 alone ($Immune_a$). Adoptive transfer of $1.0–1.2 \times 10^7$ cells was performed in mice with 3-day established MCA 105 or MCA 106 pulmonary metastases. IL-2 was administered for six doses (7500 U) ip twice daily after adoptive transfer. In Experiment 3, mice were given 500 R whole body irradiation prior to tumor inoculation.

[b] $P < 0.05$ compared to Group 2.

[c] $P < 0.05$ compared to Group 5.

immunized syngeneic mice, either freshly harvested or cultured in IVS, displayed exquisite specificity against the MCA 105 and MCA 106 tumors. These findings are in accordance with previous documentation that chemically induced tumors express distinct transplantation antigens.

Cross-reactivity of Adoptive Immunotherapy with Lymph Node Cells from Tumor-Bearing Hosts Secondarily Stimulated by IVS

In an effort to generate therapeutic effector cells from hosts bearing large growing tumors, lymphoid cells from draining lymph nodes were cultured and expanded by the IVS method. Mice inoculated with the MCA 105 or MCA 106 tumor sc in the footpad developed progressively growing tumors with a median survival time of 23 days. On Day 14 of tumor growth, when the average size of tumor reached approximately 10 mm in thickness, draining popliteal lymph nodes were harvested. These cells were cultured in IVS and were subsequently used for assessment of antitumor reactivity in adoptive immunotherapy experiments. Since lymph node cells from tumor-bearing mice do not have antitumor efficacy before IVS (10), we cannot assess the specificity of these cells.

In Table 4, MCA 106 tumor-bearing lymph node cells after IVS demonstrated significant therapeutic efficacy to MCA 106 pulmonary metastases. However, these IVS tumor-bearing lymph node cells displayed significant cross-reactivity against the antigenically distinct MCA 105 tumor. We also examined the immunologic specificity of antitumor reactivity mediated by the transfer of IVS cells generated from lymphoid cells of mice bearing MCA 105 tumors. In Table 5, MCA 105 tumor-bearing lymph node cells after IVS demonstrated similar cross-reactivity against both the MCA 105 and the MCA 106 tumors. Normal mesenteric lymph nodes identically

TABLE 4

Cross-reactivity of Adoptive Immunotherapy Mediated by IVS Lymph Node Cells from MCA 106 Tumor-Bearing Mice

Cells[a]	Lung tumor	Mean no. of metastases (SEM)		
		Expt 1	Expt 2	Expt 3
1. —	MCA 106	133 (42)	176 (8)	250
2. Normal	MCA 106	92 (50)	—	—
3. TB LN	MCA 106	11 (8)[b]	17 (3)[b]	98 (25)[b]
4. —	MCA 105	171 (28)	66 (12)	227 (23)
5. Normal	MCA 105	110 (42)	—	—
6. TB LN	MCA 105	49 (26)[c]	13 (3)[c]	54 (33)[c]

[a] Cells were harvested from draining popliteal lymph nodes (TB LN) of mice inoculated with 10^6 MCA sc in the footpad 14 days earlier and placed in IVS. Normal mesenteric lymph node cells placed in IVS were used as a control. Adoptive transfer of 2×10^7 cells was performed in mice with 3-day established MCA 106 or MCA 105 pulmonary metastases. IL-2 was administered for six doses (7500 U) ip twice daily after adoptive transfer.

[b] $P < 0.05$ compared to Group 1.

[c] $P < 0.05$ compared to Group 4.

cultured in the IVS procedure did not demonstrate any antitumor effects, indicating that the progressive tumor elicits the host immune response.

The cross-reactivity demonstrated with IVS cells from MCA 105 or MCA 106 tumor-bearing mice may have been attributed to the nonspecific LAK cell phenome-

TABLE 5

Cross-reactivity of Adoptive Immunotherapy Mediated by IVS Lymph Node Cells from MCA 105 Tumor-Bearing Mice

Cells[a]	Lung tumor	Mean no. of metastases (SEM)		
		Expt 1	Expt 2	Expt 3
1. —	MCA 105	171 (28)	66 (12)	227 (23)
2. Normal	MCA 105	110 (42)	—	—
3. TB LN	MCA 105	<1 (1)[b]	1 (1)[b]	2 (2)[b]
4. —	MCA 106	133 (42)	176 (8)	250
5. Normal	MCA 106	92 (50)	—	—
6. TB LN	MCA 106	22 (8)[c]	13 (5)[c]	175 (34)[c]

[a] Cells were harvested and placed in IVS from popliteal draining lymph nodes (TB LN) of mice inoculated with 10^6 MCA 105 sc in the footpad 14 days earlier. Normal mesenteric lymph node cells placed in IVS were used as a control. Adoptive transfer of 2×10^7 cells was performed in mice with 3-day established MCA 105 or MCA 106 pulmonary metastases. IL-2 was administered for six doses (7500 U) ip twice daily after adoptive transfer.

[b] $P < 0.05$ compared to Group 1.

[c] $P < 0.05$ compared to Group 4.

TABLE 6

Adoptive Immunotherapy of MCA 106, MCA 101, and MCA 102 Pulmonary Metastases with IVS Lymph Node Cells from MCA 106 Tumor-Bearing Mice

Cells[a]	IL-2[b]	Lung tumor	Mean no. of metastases (SEM)
1. —	−	MCA 106	250
2. —	+	MCA 106	250
3. TB LN	+	MCA 106	47 (21)[c]
4. —	−	MCA 101	250
5. —	+	MCA 101	250
6. TB LN	+	MCA 101	250
7. —	−	MCA 102	145 (8)
8. —	+	MCA 102	155 (19)
9. TB LN	+	MCA 102	164 (29)

[a] Cells were harvested from MCA 106 tumor-bearing mice and cultured in IVS as described in Table 4. Adoptive transfer of 2×10^7 cells was performed in mice with 3-day established MCA 106, MCA 101, or MCA 102 pulmonary metastases.

[b] IL-2 was administered for six doses (7500 U) ip twice daily after adoptive transfer.

[c] $P < 0.05$ compared to Groups 1 and 2.

non. LAK cells, generated by incubating normal lymphoid cells with IL-2, have been functionally defined as lymphoid cells which display nonspecific cytolytic activity to fresh, NK-resistant tumors (13). These cells have been shown to be capable of mediating the regression of 3-day established pulmonary metastases when administered in conjunction with IL-2 in a nonspecific manner (14, 15). Therefore, the degree of observed cross-reactivity was examined by using the LAK-sensitive MCA 101 and MCA 102 tumors. In Table 6, IVS cells generated from MCA 106 tumor-bearing mice mediated the regression of 3-day established MCA 106 pulmonary metastases but not MCA 101 or MCA 102 metastases. It became clear that the cross-reactivity between IVS cells derived from MCA 105 and MCA 106 tumor-bearing hosts was not due to LAK cells. More likely, antigens common to both the MCA 105 and MCA 106 tumors accounted for the cross-reactivity.

Therapeutic Efficacy of IVS Cells from MCA 106 Tumor-Bearing Hosts in the Treatment of Advanced Metastases

The observation that antigens other than tumor-specific rejection antigens can serve as target molecules for immunotherapy is interesting. In order to extend these findings, we attempted to treat advanced visible metastases with IVS cells generated from MCA 106 tumor-bearing mice. Table 7 summarized two experiments where mice with 10-day established pulmonary metastases were treated with IVS cells. As can be seen in both experiments, the adoptive transfer of $1.2–2.4 \times 10^7$ IVS lymph node cells caused the significant regression of advanced tumors.

DISCUSSION

Employing the weakly immunogenic MCA 105 and MCA 106 murine sarcomas, we have demonstrated that the regression of established pulmonary metastases could

TABLE 7

Adoptive Immunotherapy of 10-Day MCA 106 Pulmonary Metastases with IVS Lymph Node Cells from MCA 106 Tumor-Bearing Mice

Cells[a]	IL-2[b]	Mean no. of metastases[c] (SEM)	
		Expt 1	Expt 2
1. —	—	250	189 (29)
2. —	+	250	156 (30)
3. 2.4×10^7 normal	+	234 (8)	83 (29)
4. 2.4×10^7 TB LN	+	35 (10)[d]	9 (2)[d]
5. 1.2×10^7 TB LN	+	112 (35)[d]	55 (20)[d]
6. 0.6×10^7 TB LN	+	234 (12)	121 (32)
7. 0.3×10^7 TB LN	+	219 (8)	198 (33)

[a] Mice were inoculated with 10^6 MCA 106 id and inguinal lymph node cells were harvested 14 days later for IVS (TB LN). Mesenteric lymph node cells from normal mice were used for control cells in IVS. IVS cells were adoptively transferred into mice that had been inoculated iv with MCA 106 tumor cells 10 days earlier.

[b] IL-2 was administered for six doses (7500 U) ip twice daily after adoptive transfer.

[c] Pulmonary metastases were harvested 18–20 days after tumor inoculation.

[d] $P < 0.05$ compared to Group 2.

be mediated by the transfer of several different effector cell populations. Tumor regression mediated by each effector cell population displayed distinct immunologic characteristics with respect to the specificity of the antitumor reaction.

We have previously reported that the *in vivo* immunization of these tumors, which involved inoculation with viable tumor admixed with *C. parvum,* resulted in specific immunity to subsequent tumor challenges (6). Freshly harvested splenocytes from MCA 105- and MCA 106-immunized animals demonstrate the specific regression of established sc tumors. In this report, we have extended these observations to show that fresh immune splenocytes also mediated the immunologically specific regression of pulmonary metastases in criss-cross experiments.

We have also described an IVS procedure which allowed the secondary stimulation and proliferation of MCA 105 immune splenocytes with irradiated tumor cells and IL-2 (8). These immune IVS cells were found to have increased *in vivo* antitumor reactivity compared to fresh immune cells. In this report, we demonstrated that MCA 106 immune cells can also be stimulated and expanded by the IVS method. These IVS immune cells mediated potent therapeutic effects in the treatment of established pulmonary metastases. The requirements for this IVS included IL-2 and stimulation by irradiated tumor cells. Without *in vitro* tumor stimulation these cells proliferated in IL-2 but had no *in vivo* antitumor activity at the cell doses used. Similar to freshly harvested immune splenocytes, we found that the regression of 3-day pulmonary metastases caused by MCA 105 and MCA 106 immune IVS splenocytes was immunologically specific.

Until recently, previously documented reports of successful adoptive immunotherapy of established tumors have involved the use of lymphoid cells from hyperimmunized syngeneic animals. Therefore, a major problem in developing cellular immuno-

therapy for clinical applications is the generation of lymphoid cells from patients with antitumor reactivity *in vivo*. With the MCA 105 tumor we have reported the ability to generate therapeutically effective lymphoid cells after IVS from the lymphoid cells of mice bearing progressively growing MCA 105 tumor (10). The lymphoid cells were not therapeutically active when transferred before culture and required secondary stimulation by IL-2 and tumor in IVS to become active effector cells. Cells from tumor-bearing hosts which are capable of differentiating into therapeutic effector cells in IVS have been named preeffector cells. In this report, we have documented that preeffector cells are also elicited in mice bearing the antigenically distinct MCA 106 tumor. Lymph node cells from these animals mediated significant regression of 3-day and advanced 10-day pulmonary metastases after IVS. In contrast to immune IVS cells, IVS lymph node cells from MCA 105 and MCA 106 tumor-bearing hosts displayed considerable cross-reactivity against MCA 106 and MCA 105 tumors.

Because the IVS culture system employed the use of 1000 U/ml of recombinant IL-2, it is likely that the generation of nonspecific LAK cells occurred. LAK cells have been functionally defined as lymphoid cells which have been incubated in IL-2 and demonstrate nonspecific cytolytic activity to fresh, NK-resistant tumors in short-term ^{51}chromium release assays (13). In many previous experiments, LAK cells demonstrated therapeutic effects against a variety of transplantable tumors when given in large doses in conjunction with IL-2 (14, 15). However, several lines of evidence indicate that the antitumor reaction mediated by IVS cells from tumor-bearing mice was distinct from that by LAK cells. First, normal cells after IVS, which demonstrate nonspecific LAK-like cytotoxicity *in vitro,* do not have any *in vivo* therapeutic effects in the cell doses used in our experiments. The adoptive immunotherapy with LAK cells in this model requires a minimum of 2×10^8 cells to obtain consistent antitumor effects (14). Second, the cross-reactivity of tumor-bearing effectors after IVS was confined to the MCA 105 and MCA 106 tumors. These IVS cells did not mediate the regression of MCA 101 or MCA 102 tumor which are susceptible to LAK cells (15). Third, the generation of IVS cells required the sensitization of the host by the growing tumor while LAK cells can be generated from normal lymphoid cells. Last, the generation of IVS cells required additional stimulation by the tumor cells during culture whereas IL-2 alone is sufficient for LAK cell production (16). Therefore, we conclude that the cross-reactivity demonstrated by the IVS cells generated from tumor bearers was not due to the transfer of LAK cells. It is conceivable that there are common antigens shared by MCA 105 and MCA 106 tumors which are not present on MCA 101 and MCA 102 tumors and account for the observed cross-reactivity.

The difference in specificity among IVS cells generated from immunized versus tumor-bearing mice may be related to a difference in tumor antigen(s) recognition. If this is the case, the cross-reactivity may reflect the presence of common antigens other than individual tumor-specific rejection antigens on the MCA 105 and MCA 106 tumor cells. Different circumstances of antigen presentation may affect the type of immune response elicited in animals. More importantly, the demonstration of cross-reacting antigens which may serve as target molecules for immunotherapy redefines the potential of T cell immunity against cancer.

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