Specific Induction of Local Antitumor Effector Cells Mediated in Vivo by the Circulating Lymphocyte Pool

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ABSTRACT

Wistar rats are specifically resistant to the growth of the chemical carcinogen induced syngeneic tumors, Mc7 and Mc107 sarcoma, after being immunized with s.c. implants of irradiated tumor tissue. The central lymph of such immunized rats contains cells able to systemically transfer resistance against tumor growth to normal or irradiated recipient rats. The thoracic duct lymphocytes (TDL) from tumor immune donors are not directly cytotoxic against tumor targets. However recipients of i.v. infused immune TDL develop cytotoxic activity in the peritoneal cavity when challenged with the immunizing tumor at that site. Although the induction of maximum cytotoxicity is tumor specific, peritoneal lavage cells are cytotoxic against both Mc7 and Mc107 tumor targets in the 51Cr release assay. Inhibition of cytotoxicity in the assay by addition of unlabeled Mc7 or Mc107 sarcoma cells to labeled tumor targets suggests that there is both specific and nonspecific activity in these peritoneal lavage cells. Resistance to in vivo tumor growth in adoptively immunized recipients of TDL challenged with both Mc7 and Mc107 is specific for the immunizing tumor. However growth of a mixture of Mc7 and Mc107 sarcoma cells is inhibited in recipients of immune TDL. The results support the notion that mediator lymphocytes circulate in tumor immunized rats in a noncytotoxic state, specifically recognize tumor cells at a challenge site, and mediate induction of effector cells locally. These effectors are at least in part nonspecific in their cytotoxic activity.

INTRODUCTION

Although studies of immunity to experimental tumors are numerous, it is unclear what mechanisms dominate in limiting tumor growth in vivo. Cytotoxic T-cells can be generated in response to tumor antigens (1, 2), and have been found infiltrating tumor masses undergoing regression (3, 4). Tumor resistance appears to depend upon generation of this class of cells in some experimental tumor systems (5). NK-cells have been demonstrated to limit growth of metastases in mouse (6, 7) and rat (8) tumor model systems. However in some other studies, there is a poor correlation between systemic generation of lymphoid cells with cytotoxic properties and the level of resistance to tumor growth by the cell donors (9–11). Furthermore, lymphoid populations effective in systemic adoptive transfer of tumor resistance to normal recipients often lack cytotoxic activity (12–16) suggesting that interaction with other cell populations may be necessary to inhibit tumor growth.

Previous studies of the methylcholanthrene-induced Mc7 sarcoma in syngeneic Wistar rats have demonstrated that the circulating lymphocyte pool from tumor immunized animals contains cells capable of mediating specific tumor resistance (17, 18). These cells were identified as nonmitotic T-lymphocytes with a functional lifespan of at least 2 weeks. Activity was present within both “helper” and “nonhelper” phenotypic subpopulations identified by mouse monoclonal antibodies W3/25 (19) and OX-8 (20), respectively.

The present investigation extends the evaluation of the role of cells within the circulating lymphocyte pool in the expression of tumor resistance. The studies support the hypothesis that mediator lymphocytes circulate in a state lacking direct cytotoxicity. However, these lymphocytes specifically interact with tumor cells locally to generate cytotoxic effectors that can act to retard the in vivo growth of tumor cells at the challenge site. The ultimate effector cytotoxic activity is, at least in part, nonspecific.

MATERIALS AND METHODS

Animals. Wistar rats used in this study were purchased from the Trudeau Institute, Inc., Saranac Lake, NY, and were free of common rodent viruses and Mycoplasma pulmonis. The Wistar colony was derived from a breeding nucleus that was a gift from Professor R. W. Baldwin, University of Nottingham, England, and has been maintained by single line brother-sister mating. Animals in the breeding nucleus were direct descendants of the rats in which the tumors used in this study originally arose.

Tumors. The methylcholanthrene induced sarcomas Mc7 (21) and Mc107 (22) were also obtained from Professor Baldwin. Tumor tissue was stored at −80°C and passaged in normal female rats by s.c. implantation.

Preparation of Tumor Cells. Suspensions of tumor cells for challenges and cytotoxicity assays were prepared by mincing and trypsinizing tumors (23) grown s.c. in normal female rats for 10 to 18 days. Cells to be used for cytotoxicity assays were incubated 1 to 2 h at 37°C on plastic surfaces to diminish contaminating intratumor macrophages. Viability of cells determined by exclusion of trypan blue varied between 90 and 98%.

Total Body Irradiation of Rats. All rats to be used in a single experiment were placed in a confining container and treated simultaneously with 250-rad X-rays midplane dose top to bottom followed by 250-rad X-rays from bottom to top on a 4-Mev linear accelerator therapy machine in the Radiation Therapy Department of the Cleveland Veterans Administration Medical Center.

Induction of Tumor Resistance. Resistance against tumor growth was induced in rats 16–24 weeks of age by implanting s.c. into the flank, solid fragments (3 x 3 x 5 mm) of tumor tissue that had been irradiated with 12,000 rads from a cobalt-60 source at 1,300 rads/min. Each animal received two implants at weekly intervals. One week after the second implant, prospective cell donors were given 3 × 106 nonirradiated tumor cells by s.c. injection into the flank and were able to reject this tumor challenge.

Collection of TDL. A cannula was inserted into the thoracic duct of donor rats 3 or 4 days after tumor challenge and the lymph collected for 42 to 66 h. Lymph collection and TDL sedimentation and storage for the duration of the lymph collection were carried out as previously detailed (17, 18).

Collection of Peritoneal Lavage Cells. To collect cells that are found at the site of a tumor challenge, recipients of TDL were injected i.p. with 2 x 106 tumor cells and then 4 days later the peritoneal cavities were lavaged with 15–20 ml ice cold Hank's balanced salt solution with 1 unit heparin/ml. The PLC were sedimented by centrifugation at 500 x g for 10 min at 4°C, washed twice with Hank's balanced salt solution with 1% FCS and resuspended in RPMI 1640 with 10% FCS, anti-
biotics (penicillin 100 units/ml, amphotericin 0.25 µg/ml, streptomycin 100 µg/ml) and 2 × 10⁻³ M 2-mercaptoethanol at appropriate concentrations for cytotoxicity assays.

Assay of in Vivo Tumor Resistance. TDL were infused i.v. into groups of syngeneic female rats 16–24 weeks of age, 24 h after treatment with 500-rad TBI. Within 14 h of cell transfer, 1 × 10⁵ to 2 × 10⁶ tumor cells in 0.1 ml were injected into the calf muscle of the hind legs of test and control animals. Serial caliper measurements were made of the anterior-posterior and medial-lateral diameters of the legs at the injection sites. The increase in the average leg diameter over baseline values provided an objective measurement of tumor growth in each rat. The difference between tumor sizes in test and appropriate control groups was taken as the level of tumor resistance of the test group reflecting the activity of the transferred TDL.

Cytotoxicity Assay. Mc7 and Mc107 sarcoma cells were labeled with chromium-51 by incubating 2 × 10⁶ cells suspended in 1-ml RPMI 1640 with 10% FCS, antibiotics and 2 × 10⁻³ M 2-mercaptoethanol containing 100 µCi Na²⁶CrO₄ (575 mCi/mg; New England Nuclear, Boston, MA) at 37°C for 90–120 min. The cells were then washed three times and diluted with the same medium to a concentration of 1 × 10⁷/ml. One hundred µl of the medium containing 1 × 10⁶ labeled tumor targets was dispensed into flat bottomed wells of 96 well tissue culture plates (Linbro Scientific, Inc., Hamden, CT). Lymphoid cells at appropriate concentrations, in the same medium resulting in effector:target ratios of 160:1 to 20:1, or medium only to determine spontaneous release, or 1% sodium dodecyl sulfate to determine total release, were added to appropriate wells. An additional 100 µl of medium or in some experiments, culture supernatants, or unlabeled tumor cells were added to wells. Final volume in all wells was 300 µl. Plates were incubated at 37°C in a moist 5% CO₂ atmosphere for 6 h. Then 100 µl of supernatant was removed from each well, transferred to plastic tubes, and counted in a γ counter. All lymphoid cells were tested in triplicate. Spontaneous and total release were each tested in 12 replicate wells. Cytotoxic activity was quantitated as the percentage of specific chromium-51 release:

\[
\text{Cytotoxicity} = \frac{\text{experimental release cpm} - \text{spontaneous release cpm}}{\text{total release cpm} - \text{spontaneous release cpm}} \times 100
\]

Inhibition of cytotoxicity by unlabeled tumor cells was quantitated as percentage of inhibition:

\[
\text{Cytotoxicity PLC only} - \text{cytotoxicity PLC + unlabeled tumor cells} \times 100 = \text{Cytotoxicity of PLC only}
\]

Statistical Analysis. Tumor sizes in different groups were compared by the nonparametric sum of ranks test (24). Values of cytotoxicity demonstrated by test and control cells were compared by unpaired t tests.

RESULTS

Effect of TBI upon Expression of Resistance to Tumor Growth in Vivo Mediated by the Circulating Lymphocyte Pool. It has been previously demonstrated that TDL obtained from Mc7 sarcoma immunized Wistar rats contained T-cells able to mediate resistance against tumor growth when systemically transferred to normal recipient rats (17). It has been suggested that irradiated recipients are better subjects for adoptive transfer studies (13). To determine whether TDL could mediate protective tumor immunity in recipients exposed to total body irradiation, as has been demonstrated for other lymphoid populations such as spleen cells (13, 15, 16), groups of rats were treated with 500-rad TBI, infused i.v. with Mc7 immune TDL, normal TDL, or medium only, and then challenged with i.m. injections of Mc7 sarcoma cells. The results of a representative experiment are shown in Fig. 1. Tumor sizes in the recipients of TDL from Mc7 immunized donors were significantly smaller (P < 0.05) than tumors in recipients of normal TDL or medium only at 2, 3, and 4 weeks after the challenge injection. There was no significant difference between tumor growth in the latter two groups. These data indicate that radiosensitive accessory cells outside the TDL population were not mandatory for the expression of in vivo resistance to tumor growth. Furthermore, just as in nonirradiated recipients (17), transfer of normal TDL failed to alter growth of Mc7 sarcoma indicating that recipients of medium only are suitable controls for the adoptive transfer of resistance assay.

Transfer of the Induction of Cytotoxic Cell Populations. Although TDL from tumor immunized rats were able to systemically transfer resistance against tumor growth, this population failed to demonstrate cytotoxic activity against tumor targets in a 6-h chromium-51 release assay, even when enriched subpopulations were tested at effector to tumor ratios of 200:1 (data not shown). To test the possibility that transferred TDL were responsible for generation of cytotoxic activity at the site of tumor challenge, TDL were obtained from normal or Mc7 immunized donor rats and infused i.v. into normal rats or rats exposed to 500-rad TBI 24 h prior to cell transfer. TDL recipients were challenged by injecting Mc7 cells into the peritoneal cavity rather than into the leg. Four days later, the peritoneal cavities were lavaged to recover the cellular populations that were present. These peritoneal lavage cells were pooled within groups and tested for cytotoxicity against Mc7 target cells using the 6-h ⁵¹Cr release assay. Fig. 2 shows that higher levels of cytotoxicity were present in the PLC from recipients of Mc7 immune TDL compared to PLC from recipients of normal TDL. The PLC cytotoxic activity from nonirradiated and irradiated Mc7 adoptively immunized rats was the same on a cell for cell basis. Thus, cytotoxic activity was present.

Fig. 1. Growth of Mc7 sarcoma in irradiated recipients of TDL. All rats were exposed to 500-rad TBI 24 h prior to i.v. infusion of 5 × 10⁶ Mc7 immune TDL, 5 × 10⁶ normal TDL or medium only. All rats were challenged with 1 × 10⁶ Mc7 sarcoma cells i.m. 14 h after i.v. TDL. Points, median tumor diameters; bars, tumor diameter ranges in mm, five rats per group; 0, tumor sizes in recipients of Mc7 immune TDL; ×, tumor sizes in recipients of normal TDL; †, tumor sizes in recipients of medium only. Recipients of Mc7 immune TDL show significantly smaller tumor sizes than recipients of normal TDL or medium only at 2, 3, and 4 weeks after challenge (P < 0.05). There is no significant difference in tumor sizes between normal TDL and medium only recipients.
outside the transferred TDL were not mandatory for the approach i.p. with Mc7 and radiosensitive accessory cells of normal TDL challenged i.p. with the same tumor. The possibility was tested that cytotoxic cells were appearing in the peritoneal cavities of recipients of Mc7 immune TDL recipients challenged i.p. with the tumor different from that used to immunize the TDL donors. PLC from recipients of irradiated TDL recipients had the highest cytotoxic activity when the i.p. challenge was the same as the tumor used to immunize the TDL donors. PLC from recipients challenged i.p. with the tumor different from that used to immunize TDL donors had levels of cytotoxicity similar to those of PLC from recipients of normal TDL challenged with the same tumor. Thus eliciting the peritoneal cytotoxic activity was tumor specific. However, the PLC demonstrated no apparent in vitro tumor specificity since there was activity against both Mc7 and Mc107 tumor targets.

In Vivo Specificity of PLC Cytotoxic Activity. To further examine the cytotoxic specificity of the specifically induced PLC, unlabeled Mc7 or Mc107 sarcoma cells were added to the labeled tumor targets in the $^{51}$Cr release assay. Fig. 3 shows that in three such experiments, both cold targets are capable of inhibiting labeled target lysis. However, unlabeled immunizing tumor cells more efficiently inhibited the cytotoxic activity of PLC against immunizing tumor targets (Fig. 3, A and D). In contrast, the cytotoxic activity of PLC against labeled tumor targets not involved in immunization of the donors was similarly inhibited by the same numbers of added unlabeled Mc7 or Mc107 sarcoma cells (Fig. 3, B and C). These observations support the idea that PLC contain both specific and nonspecific effector cells.

In other experiments, supernatants from specifically induced PLC cultured with unlabeled Mc7 or Mc107 sarcoma cells at an 80:1 ratio, were not directly cytotoxic against tumor targets. Furthermore, these supernatants were not able to enhance levels of cytotoxicity of PLC against Mc7 or Me107 cells (data not shown). These results suggest that the PLC and not soluble factors generated and released during incubation were the effectors in the assay.

Specifity of Resistance against in Vivo Growth of Mc7 and Mc107 Sarcomas Mediated by TDL. The observation that specifically induced PLC from tumor immune donors had cytotoxic activity against both Mc7 and Mc107 sarcoma also raised the question whether the tumor targets tested have shared antigeneity. However with regard to in vivo tumor growth, Mc7 and Mc107 sarcomas did not appear to be cross-protective in

### Table 1

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<td>i.v. TDL infused</td>
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- a Irradiated 500-rad TBI. Peritoneal cavities lavaged 4 days after i.p. tumor challenge. Three rats per group.
- b Mean ± SD of triplicate values of specific chromium-51 release from labeled tumor targets incubated with PLC.
- c $5 \times 10^6$ TDL infused i.v. 24 h after TBI.
- d $2 \times 10^6$ tumor cells injected i.p. 4 h after i.v. TDL.
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Specificity of Induction of Cytotoxic PLC Mediated by TDL. The possibility was tested that cytotoxic cells were appearing in the peritoneal cavities of recipients of TDL as a result of the nonspecific stimulus of i.p. tumor cell injection. TDL drained from Mc7 sarcoma immune, Mc107 sarcoma immune, and normal donors were infused into groups of rats treated with 500-rad TBI. Twenty-four h later half of each group was challenged i.p. with Mc7 and the other half with Mc107 sarcoma cells. Four days later PLC were obtained, pooled within groups, and tested for cytotoxic activity against both Mc7 and Mc107 tumor targets. The results of a representative experiment are shown in Table 1. PLC from irradiated TDL recipients had the highest cytotoxic activity when the i.p. challenge was the same as the tumor used to immunize the TDL donors. PLC from recipients challenged i.p. with the tumor different from that used to immunize TDL donors had levels of cytotoxicity similar to those of PLC from recipients of normal TDL challenged with the same tumor. Thus eliciting the peritoneal cytotoxic activity was tumor specific. However, the PLC demonstrated no apparent in vitro tumor specificity since there was activity against both Mc7 and Mc107 tumor targets.

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ADOPTIVELY TUMOR IMMUNIZED RATS. IN Table 2, growth of each tumor is compared in irradiated recipients of MC7 immune, MC107 immune, or normal TDL. All animals were challenged i.m. in the right hind leg with MC107 and in the left hind leg with MC7 sarcoma cells. Sizes of MC107 tumors in recipients of MC107 immune TDL (group 1) were significantly smaller than in recipients of MC7 immune or normal TDL (P < 0.05). The MC107 tumor sizes in the latter two groups were not different. The opposite was true in the recipients of MC7 immune TDL (group 2). Sizes of the MC7 tumors in the left hind legs were smaller than in MC107 immune TDL (group 1) or normal TDL (group 3) recipients (P < 0.05). Sizes of MC7 tumors in the latter two groups were similar. These results demonstrate not only the specificity of tumor immune TDL in mediating resistance to tumor growth, but also indicate that the specific tumor challenge in recipients of immune TDL did not result in the in vivo generation of nonspecific effectors capable of altering the growth of the tumor not involved in immunization implanted in another site.

RESISTANCE TO IN VIVO GROWTH OF MIXED TUMOR CHALLENGES MEDIATED BY IMMUNE TDL. Although recipients of immune TDL demonstrated specific resistance to the growth of a tumor challenge and generated cytotoxic cell populations at the site of specific tumor challenge, the specifically induced PLC had both specific and nonspecific activity in vitro. To test whether local induction of nonspecific effectors might contribute to in vivo tumor resistance, the growth of mixed tumor challenges in irradiated adoptively immunized rats was measured. Fig. 4 demonstrates the results of two similar experiments. In experiment 1, TDL from MC107 immune donors or medium only were infused into two respective groups of rats previously exposed to 500-rad TBI. One half of each group was challenged i.m. in the hind leg with 1.0 x 10^5 MC7 sarcoma cells and the other half with the same number of MC7 cells mixed with 1.5 x 10^5 MC107 sarcoma cells. Tumor sizes were measured at weekly intervals. The growth of MC7 cell challenge in recipients of MC107 immune TDL or medium only did not differ significantly indicating the lack of resistance mediated by MC107 immune TDL against i.m. implanted MC7 cells. MC7 plus MC107 cell challenge in recipients of medium only also grew in similar fashion, indicating that mixing the two tumors to give a higher density of sarcoma cells in the inoculum did not inhibit growth of the tumor challenge. However the recipients of MC107 immune TDL challenged with the MC7 plus MC107 cell mixture had significantly smaller tumors (P < 0.01) than the other three groups at 3, 4, and 5 weeks after tumor challenge.

In experiment 2, irradiated recipients received TDL from

| Table 2. Specificity of resistance against the growth of tumor challenge mediated by TDL |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Group           | Treatment^        | Growth of challenge—median (range)^4 |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| 1               | MC107 Immune TDL IV | 6.5 (4.0-9.0)^5  |
| 2               | MC7 Immune TDL IV  | 10.0 (9.0-11.5)  |
| 3               | Normal TDL IV     | 12.0 (9.0-14.0)  |
|                 |                   | 9.0 (6.5-10.0)   |
|                 |                   | 3.5 (3.0-5.0)^6  |
|                 |                   | 9.0 (5.5-10.0)   |

* All exposed to 500-rad TBI 24 h prior to 4 x 10^6 TDL i.v.
* All challenged with 1.5 x 10^5 MC107 sarcoma cells i.m. in the right hind leg and 1.0 x 10^5 MC7 sarcoma cells i.m. in the left hind leg 24 h after TDL infusion. Diameters of tumors in millimeters 20 days after challenge. Groups of five rats.
* P < 0.05 tumor sizes smaller than same challenge in recipients of other immune TDL and normal TDL.
* Obtained from donors immunized against MC107 sarcoma.
* Obtained from donors immunized against MC7 sarcoma.
* Obtained from unimmunized donors.

DISCUSSION

The present study further examines the mechanisms of resistance against in vivo growth of MC7 sarcoma in syngeneic Wistar rats. In this model, rats made resistant to tumor growth by implantation of irradiated tumor tissue have T-cells within their central lymph that are able to systemically mediate specific
Resistance to tumor growth in normal (17) or irradiated recipients (present study). Utilizing adoptive transfer of resistance as an assay of in vivo activity, mediator cells have been shown to belong to both helper and nonhelper phenotypes (18). However, there is a lack of demonstrable in vitro cytotoxicity against Mc7 sarcoma cells by TDL, even when T-cell subpopulations enriched in nonhelper or helper phenotypes are assayed at high lymphoid:tumor target ratios. This observation could reflect a total lack of active cytotoxic cells in TDL or numbers that are too low to be detected by the assay. It is therefore unlikely that cells circulating in an actively cytotoxic state within the TDL population are the major effectors of in vivo tumor resistance. A more likely explanation is that circulating sensitized lymphocytes are mediator cells lacking cytotoxic potential but able to direct generation of cytotoxic effectors and/or are precursors of cytotoxic effector cells at the time they are delivered to the blood from central lymph in tumor immunized rats.

The lack of cytotoxicity in tumor immune TDL is consistent with numerous other studies that demonstrate tumor resistance can be transferred with noncytotoxic cells obtained from a variety of lymphoid compartments (11–16). It should be stressed however, that the TDL differ from other lymphoid compartments such as spleen or lymph node, utilized in most other adoptive transfer systems. Since drainage of the thoracic duct intercepts lymph flow from entry into venous blood, all TDL recovered are destined to enter the venous circulation. The TDL infused i.v. into recipient assay animals would be expected to circulate and function as if in the original donor. Therefore, expression of tumor resistance in TDL recipients should reflect the physiological activity of the circulating lymphocytes. In contrast, other lymphoid compartments may contain cells that do not readily enter the circulation. Yet these populations have been placed into the circulation of recipients and may populate specific compartments or directly influence tumor growth. However it is unclear whether this potential difference in tissue distribution influences the transfer of tumor resistance. TDL also differ functionally from spleen cells, the most frequently used population in adoptive transfer studies. The spleen contains substantial NK-cell activity whereas rat TDL lack this function (25).

Tumor immune TDL are able to mediate not only systemic resistance to tumor growth, but also the appearance of cytotoxic cells in the peritoneal cavity when recipients are challenged at that site with tumor cells. The induction of increased levels of cytotoxic activity after i.p. tumor injection is specific for the presence of immunizing tumor in the inoculum. Intraperitoneal injection of the sarcoma cells not used to immunize TDL donors resulted in similar low levels of cytotoxicity in both recipients of tumor immune and normal TDL. Comparable modest levels of cytotoxicity have been found in washouts of nonstimulated peritoneal cavities of normal rats or rats bearing some syngeneic tumors. This native or unstimulated cytotoxic activity has been attributed to macrophages and NK-cells (26).

Although the induction of maximum cytotoxicity in the peritoneal cavity is specific, the PLC show cytotoxic activity against both Mc7 and Mc107 tumor targets in the chromium-51 release assay. The production and release of soluble factors during the assay do not appear to influence levels of cytotoxicity. Inhibition patterns with the addition of unlabeled tumor cells to labeled tumor targets in the assay (Fig. 3) suggest that both specific and nonspecific components make up the cytotoxic activity and therefore the effectors are likely heterogeneous. These have not yet been identified but possible candidates include specific (4, 5) and nonspecific (27, 28) cytotoxic T-lymphocytes, lymphokine activated killer cells (29, 30), NK-cells (31), macrophages (32, 33), or neutrophils (34).

Even though both Mc7 and Mc107 sarcoma targets are susceptible to in vitro injury by either specifically induced PLC, the in vivo i.m. growth of each tumor is specifically inhibited in recipients of tumor immune TDL (Table 2). These results demonstrate that Mc7 and Mc107 sarcomas are antigenically different and the initiation of antitumor activity by tumor immune TDL is specific. In addition, locally generated effector cells are unable to influence the growth of the nonimmunizing tumor challenge implanted at a distant site. However, if recipients of immune TDL are injected with a mixture of both Mc7 and Mc107 sarcoma cells, the growth of the mixed tumor challenge is inhibited (Fig. 4). These experiments demonstrate that the local induction of effector cell activity requires the presence of immunizing tumor at the challenge site and at least some of the effectors are nonspecific. A similar “bystander” effect has been found in some other but not all experimental systems examined (35–39). It has been demonstrated in at least one adoptive transfer setting (39). Conditions favoring expression of the effect include challenge with a high ratio of specific targets to bystander cells, use of syngeneic rather than allogeneic tumors, and a primary rather than anamnestic immune response in tumor challenged hosts.

In summary, in this tumor model system, it appears that lymphocytes within the circulating pool of tumor immunized rats specifically recognize tumor cells and inhibit in vivo tumor growth at a challenge site by mediating generation of effector cells locally. Although lymphocytes possessing direct cytotoxic properties are not present in appreciable numbers in the TDL, it is unclear whether precursors of the effector cells are contained within the TDL or derived from circulating cells outside the central lymph, or from a noncirculating local source. At least some of the effectors operative at the challenge site have nonspecific activity but do not circulate in adequate numbers to influence the growth of a different tumor at a distant site. These findings support the notion that specificity of the in vivo tumor resistance is at the level of recognition by the circulating mediator cells rather than at the ultimate effector cell level and implies that growth of antigenically diverse tumor masses could be inhibited in immune hosts if some of the tumor cells displayed immunizing antigen(s).

REFERENCES

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