Enhanced Suppressor Macrophage Activity Associated with Termination of the L5178Y Cell Tumor-dormant State in DBA/2 Mice

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ABSTRACT
Both cytolytic T-lymphocytes and cytolytic macrophages have been implicated in the long-term maintenance of L5178Y cells in a tumor-dormant state in DBA/2 mice. Eventually, however, the tumor-dormant state terminates, and all mice develop ascitic tumors. In an evaluation of the mechanisms involved in termination of the tumor-dormant state, we detected in the peritoneal cavity of many tumor-dormant mice macrophages with increased capacity to suppress the in vitro generation of a secondary anti-L5178Y cell cytolytic T-lymphocyte response. The incidence of macrophage-mediated immunosuppressive activity in individual tumor-dormant mice was related directly to the number of tumor cells in the peritoneal cavity of those mice. Furthermore, in tumor-dormant mice harboring fewer than 5 × 10⁴ L5178Y cells, the detection of macrophage-mediated immunosuppressive activity was a prognostic indicator of termination of the tumor-dormant state and development of an ascitic tumor. These data suggest that peritoneal macrophage-mediated immunosuppressive activity, through inhibition of cytolytic T-lymphocyte generation in vivo, contributes to the termination of the tumor-dormant state and development of ascitic tumors.

INTRODUCTION
The progressive growth of tumors in animals and humans often occurs in the presence of antitumor cytotoxic immune activity (29). These cytotoxic responses are often subverted by host suppressor cells or suppressor factors (14, 16), thereby facilitating tumor growth. Immunosuppression can be tumor specific, mediated by T-lymphocytes, or nonspecific, mediated by T- or B-lymphocytes or macrophages (14). Suppressor cells have been implicated in the induction of primary tumors (3), in the progressive growth of transplanted tumors (2, 5, 18), and in the failure of adoptive immunotherapy (2).

A unique model system for the evaluation of antitumor cytotoxic effector cells and immunoregulatory cells is the tumor-dormant state, established in DBA/2 mice with L5178Y lymphoma cells (30). DBA/2 mice which are immunized and challenged with L5178Y cells remain clinically normal for many weeks while harboring small numbers of L5178Y cells in the peritoneal cavity (27). The establishment of this tumor-dormant state is temporally associated with a strong peritoneal CTL response which peaks at 4 days post-L5178Y cell challenge, declines to background levels by 40 to 70 days postchallenge, and can be reelicited from memory T-cells by i.p. inoculation of a large dose of L5178Y tumor antigen (13). Following the decline in the initial CTL activity, tumor-dormant mice which harbor small numbers of tumor cells also contain peritoneal T-cells and macrophages which produce a synergistic cytolytic activity against L5178Y cells in vitro (22). In this synergistic effect, the cytolytic activity of combined T-cell:macrophage populations is significantly greater than the additive cytolsis produced by the individual populations. When the tumor cells proliferate to levels >10⁴ cells/tumor-dormant mouse, a direct macrophage-mediated cytolytic response can also be detected (21).

In spite of these varied cytolytic peritoneal cell responses, all tumor-dormant mice eventually terminate their tumor-dormant state and develop an ascitic tumor (27). As the mouse proceeds through the tumor-dormant state, there is a progressive selection from the heterogeneous original L5178Y cell challenge inoculum of "emergent"-phenotype L5178Y cells, which are less susceptible to all of the above peritoneal cell cytolytic responses than is the original L5178Y cell population (Refs. 21 and 22; Footnote 5). Termination of the tumor-dormant state is associated with a dominance in the peritoneal tumor population of emergent-phenotype L5178Y cells which grow out to form ascitic tumors (5). We report here that termination of the tumor-dormant state is also associated with the appearance in the peritoneal cavity of macrophages which have an enhanced capacity to subvert the generation of T-cell-mediated cytolytic activity in vitro. The incidence of suppressor macrophage activity in the tumor-dormant population is related directly to the tumor burden in the peritoneal cavity and is a prognostic indicator of termination of the tumor-dormant state and development of an ascitic tumor.

MATERIALS AND METHODS
Animals. Female DBA/2 mice were obtained at 8 weeks of age from The Jackson Laboratory, Bar Harbor, Maine.
Tumor Cells. The tumor cell lines used in this study were the methylicholanthrene-induced DBA/2 lymphoma L5178Y and the dimethylbenzanthracene-induced C57BL/6 lymphoma EL-4. These cells were maintained in suspension culture in MEM (Grand Island Biological Co., Grand Island, N. Y.), supplemented with 10% fetal calf serum, 2 mM glutamine, 1 mM sodium pyruvate, 1× nonessential amino acids, sodium bicarbonate (1500 mg/lter), gentamicin (50 µg/ml), and 15 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, and were incubated at 37° in a humidified 5% CO₂ atmosphere.
Establishment of the L5178Y Tumor-Dormant State. This procedure has been described (27). Briefly, mice receive a s.c. inoculation of 10⁶ L5178Y cells on the midventral surface. The resulting 1-cm-diameter tumor nodule is surgically excised 10 days later, and the mice are challenged i.p. with 5 × 10⁴ L5178Y cells 7 days after tumor nodule excision.

¹ This research was supported by Grant CA32577 awarded by the National Cancer Institute, Department of Health and Human Services, and by Biomedical Research Support Grant 5 S07 RO5413.
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⁴ The abbreviations used are: CTL, cytolytic T-lymphocyte(s); MEM, minimal essential medium; PBS, phosphate-buffered saline; PC, peritoneal cell(s); PPL, partial peritoneal lavage; AD, adherent; MLTC, mixed lymphocyte:tumor cell.
Received April 15, 1983; accepted September 6, 1983.

DECEMBER 1983

5831
Selection of Experimental Mice and Enumeration of Tumor Cells. At 25 days post-L5178Y challenge, all mice processed through the tumor dormancy establishment protocol underwent a partial peritoneal lavage. The mice received 2.5 ml of pyrogen-free PBS i.p. and were lightly anesthetized, the abdomens were massaged gently, and 0.4 to 1.0 ml of the PBS was withdrawn from the peritoneal cavity by needle and syringe. The PC were centrifuged and resuspended in MEM at a volume equal to the volume of PBS withdrawn. Then, 0.2 ml of each PC suspension was delivered, in duplicate, into the first well of each of 2 rows of a microtiter plate (Costar, Cambridge, Mass.), and 0.1 ml was removed and serially diluted 1:2 through 24 wells. The number of tumor cells in the 0.1 ml remaining in Well 1 was calculated after a 14-day incubation period, by noting the terminal well giving positive tumor cell outgrowth. This number of tumor cells in Well 1 was multiplied by 25 (2.5 + 0.1) to give an estimate of the tumor burden of each mouse at 25 days postchallenge. There is a 100% plating efficiency of L5178Y cells in this assay (13), and tumor cells from tumor-positive wells are tumorigenic in vivo. In the PPL technique, an absolute correlation has been established between the percentage of the inoculated volume removed and the percentage of the total PC removed in the PPL volume. Mice characterized as tumor dormant negative on the basis of this assay remain tumor negative, and >99% of mice characterized as tumor dormant (<5 x 10^4 tumor cells) eventually succumb to ascitic tumors. Tumor-dormant mice, identified by the PPL technique performed at 25 days post-L5178Y cell challenge, were selected for experiments at various times between 36 and 70 days postchallenge.

Separation of Adherent and Nonadherent PC. PC from individual mice were added to fetal calf serum-precoated tissue culture plates, the nonadherent cells were discarded, and the AD PC were recovered by a modification of the procedure described by Kumagai et al. (12). This protocol and the cellular composition of the recovered AD PC populations have been described (21).

In Vitro Generation of Cellular Cytolytic Activity in MLTC Cultures. For syngeneic MLTC cultures, responder spleen cells from tumor-dormant negative mice (see above) were teased from the spleen capsules and resuspended in MEM at 10^7 cells/ml. The stimulator cells were L5178Y cells obtained from logarithmic growth phase suspension cultures and irradiated with 8000 rads of X-irradiation (250-kV X-ray source, 2-mm aluminum filtration; Perkins Stabilipan). The MLTC cultures consisted of 10^5 responder spleen cells and 10^5 stimulator irradiated L5178Y cells (responder:stimulator ratio = 100:1) in 2.5 ml of MEM containing sodium bicarbonate (1250 mg/liter) and no N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid in 16-mm wells of 24-well tissue culture plates (Costar). AD PC were added to certain cultures as indicated in the text. After 5 days of incubation (37° in a humidified 5% CO_2 atmosphere), the cells were washed once, resuspended in MEM, and tested for cytolytic activity in the 51Cr release assay described below. Cytolytic Antiserum Treatment. Treatment of MLTC-generated cytolytic cells with anti-Thy 1.2 antibody plus rabbit complement was performed as described previously (22). The anti-Thy 1.2 (provided by Dr. T. MacDonald, Thomas Jefferson University, Philadelphia, Pa.) was used at a 1:10 dilution, the highest dilution which would completely ablate CTL-mediated cytolytic activity. Rabbit complement was used at a 1:20 dilution. For treatment of AD PC, the cells were suspended in 5 ml of MEM and split into two 2.5-ml aliquots. Then, the anti-Thy 1.2 and guinea pig complement or guinea pig complement alone was added at a final dilution of 1:10 each. The suspensions were vortexed and added to fetal calf serum-coated plates for the 90-min adherence step at 37°C in a humidified 5% CO_2 incubator. Cytolytic T-cells from the peritoneal cavity of a mouse 7 days post-L5178Y challenge (21, 27) were treated in similar fashion and served as positive control for the absence of anti-Thy 1.2 plus complement to ablate CTL activity.

Cytolytic Assay. Effector cytolytic cells from MLTC cultures were added, in 3 to 4 3-fold dilutions, to round-bottomed microtiter plates (Costar) in 0.1 ml of MEM. L5178Y cells were labeled for 3 to 4 hr in 1 ml of MEM containing 200 μCi of 51Cr (Na^51CrO_4; specific activity, 200 to 500 mCi/mg; New England Nuclear, Boston, Mass.), washed 4 times, and resuspended at 10^5 cells/ml in MEM. Then, 0.1 ml (10^5 labeled cells) was added to each cytolytic assay culture. The effector:target cell ratios were varied in each experiment from 2:1 to 60:1, based on effector cell recovery. All cytosis assay cultures were incubated at 37°C in a humidified 5% CO_2 atmosphere. After an 18-hr incubation period, 1 ml of the culture fluid was removed and counted in a well-type γ-counter (Beckman Model 800C; Beckman Instruments, Inc., Irvine, Calif.). Maximum isotope release was determined by detergent (0.5% Nonidet P-40 in PBS) lysis, and spontaneous release was determined by incubation of labeled tumor cells in medium only. The spontaneous release averaged 20 ± 5% (S.E.). The percentage of 51Cr release was calculated according to the formula.

% of 51Cr release =

\[
\frac{\text{Experimental cpm release} - \text{cpm spontaneous release}}{\text{Maximum cpm release} - \text{cpm spontaneous release}} \times 100
\]

The S.E. of triplicate cultures was <10% of the mean cpm values obtained.

RESULTS

The Anti-L5178Y Cell MLTC Culture. The immunosuppressive effects of AD PC were measured in MLTC cultures in which the responder cells were spleen cells from mice which had been immunized and then had rejected the L5178Y tumor cell challenge. These spleen cells exhibited little anti-L5178Y cell cytolytic activity in a 51Cr release assay when tested either directly from the mice (data not shown) or after 5 days in culture in the absence of irradiated L5178Y cells (Table 1). The addition of irradiated L5178Y stimulator cells to the spleen cell cultures ( responder:stimulator ratio, 100:1) resulted in the generation of a high level of cytolytic activity. The antigen-driven cytolytic response was ablated by treatment of the MLTC culture-derived cells, at the end of the incubation period, with antibody to Thy 1.2 in the presence of complement (Table 1).

Dose Effect of AD PC from Normal DBA/2 Mice on the In Vitro Generation of CTL. Spleen cells require the accessory function of AD cells to generate a CTL response in vitro (9, 24). However, this is a highly dose-dependent phenomenon, and concentrations of normal AD PC above 5% in vitro have been shown to be immunosuppressive (9, 24). As shown in Chart 1, the generation of CTL activity in MLTC cultures was inhibited by AD PC from normal DBA/2 mice at a dose of 10% and slightly enhanced by AD PC doses of 1 and 3%. Therefore, we tested AD PC at a dose of 3% to compare the immunosuppressive effects of AD PC from normal and tumor-dormant mice. The S.D.
between MLTC-generated CTL responses of replicate control cultures, within any given experiment, was approximately 10%. For this reason, we selected ≥30% (3 times S.D.) suppression of the CTL response, at an effector/target ratio within the linear portion of the percentage of 51Cr release versus effector/target ratio titration curve, as a cutoff for positive immunosuppression. At the 3% AD PC dose, there was no reduction of the viable cell recovery from the MLTC cultures by any added AD PC population (suppressive or nonsuppressive).

Correlation of Macrophage-mediated Suppression of In Vitro Generation of CTL with the Tumor Burden of Tumor-dormant Mice Supplying the Macrophages. There was no universal correlation between the degree of suppression of CTL generation in MLTC cultures by macrophages from individual tumor-dormant mice and the number of tumor cells within the peritoneal cavity of those mice. However, there was a direct correlation between the percentage of tumor-dormant mice expressing suppressor macrophage activity and the tumor burdens in those mice. As shown in Chart 2, 32% of tumor-dormant mice which contained <10^4 tumor cells also contained suppressor peritoneal macrophages, an incidence comparable to age-matched normal DBA/2 mice (29%). However, 75% of tumor-dormant mice which contained 10^4 to 10^5 tumor cells, 83% of mice with 10^5 to 10^6 tumor cells, and 100% of mice with >10^6 to 10^7 tumor cells also contained suppressor macrophage activity.

Lack of Tumor Cell-induced Suppression of In Vitro Generation of CTL. Since peritoneal cell populations from tumor-dormant mice contain L5178Y cells, it was possible that tumor cells contaminated the AD PC population and that the immunosuppressive effects produced by AD PC in MLTC cultures were caused by these tumor cells. Such effects could occur in the cytolytic assay by cold tumor cell target competition or during the CTL induction phase by tumor cell-induced suppression or suppression by soluble factors released from the tumor cells (1, 25, 26).

Several lines of evidence, however, argue against the involvement of tumor cells in the observed immunosuppression: (a) as shown in Table 2, 46% of the cultures in which suppression was observed contained no recoverable tumor cells on the day of CTL assay, and 29% contained 1 to 100 tumor cells; (b) the suppression observed in cultures containing 101 to 2,000 recoverable tumor cells was probably not due to tumor cells, because the addition of 1,000 viable L5178Y cells to MLTC cultures on Day 0 resulted in the recovery of 2,000 to 10,000 tumor cells on Day 5, yet these cultures showed no suppression of CTL activity; and (c) the addition to MLTC cultures of a 10% v/v concentration of cell-free supernatant from L5178Y cell cultures growing in suspension at 5 to 6 x 10^7 cells/ml did not suppress the resultant CTL response (data not shown). Thus, there is no evidence to indicate that AD PC-mediated suppression of the CTL response was due to the few contaminating tumor cells recovered from some of the cultures.
Evidence That Macrophages Are Mediators of AD PC-induced Suppression of CTL Generation. The AD PC populations from normal and tumor-dormant DBA/2 mice, which were recovered from serum-coated tissue culture dishes, consisted of ≥93% macrophages, ≤7% lymphocytes, and ≤1% granulocytes, based on morphology and esterase-staining characteristics (21). These cell proportions strongly suggest that macrophages produced the AD PC-mediated suppression. Lymphocytes would have had to function at a dose ≤0.2% in the MLTC cultures, which is far lower than that required in other systems (19, 20, 23). However, since adherent T-cells can contaminate AD PC populations (6, 11), we investigated the possible role of suppressor T-cells in our system. We treated AD PC from tumor-dormant mice, during the adherence procedure, with anti-Thy 1.2 antibody plus guinea pig complement or with complement alone and assayed both populations for suppression of the CTL response in MLTC cultures. As shown in Chart 3, both treated populations inhibited the CTL response to the same extent, indicating a lack of T-cell-mediated suppression and suggesting that macrophages function as the immunosuppressive cells. Since L5178Y cells also express Thy 1.2 (28), the immunosuppressive activity of the AD PC cells after treatment with anti-Thy 1.2 antibody plus complement is further evidence that contaminating tumor cells are not responsible for the immunosuppressive effects.

Specificity of the Immunosuppressive Activity of Macrophages from Tumor-dormant Mice. The antigen specificity of the immunosuppressive activity of peritoneal macrophages from tumor-dormant mice was tested. The AD PC from each of 5 tumor-dormant mice were added at a 3% dose to both syngeneic MLTC (see above sections) and allogeneic MLTC (DBA/2 anti-CS7BL/6) cultures (see "Materials and Methods"). After 4 days of incubation, the cytolytic activity of the syngeneic MLTC cultures was tested against 51Cr-labeled L5178Y cells and of the allogeneic MLTC cultures against 51Cr-labeled EL-4 cells. As shown in Chart 4, the macrophages from 3 of the 5 tumor-dormant mice did not suppress the cytolytic responses in either culture. In contrast, the macrophages from Mouse 4 and Mouse 5 suppressed the cytolytic responses in both cultures, indicating that the immunosuppressive activity of peritoneal macrophages from tumor-dormant mice is antigen nonspecific.

The immunosuppressive activity of macrophages from tumor-dormant mice was further demonstrated to be operative during the generation phase of the CTL response rather than the effector phase. Peritoneal AD cells from normal, tumor-dormant, or tumor-emergent DBA/2 mice had no inhibitory effect on fully lytic CTL when added to the cytolyis assay at doses of 10, 30, and 100% (Table 3).

Suppressor Macrophage Activity as a Prognostic Indicator of the Early Development of an Ascitic Tumor. The direct correlation between the percentage of mice expressing suppressor macrophage activity and their tumor cell burden (Chart 2) gave no indication as to whether this suppressive activity was a causative factor in tumor growth or the result of tumor growth or a combination of both. To address this question more directly, we utilized the PPL technique, which can be performed on tumor-dormant mice without killing the mice and without affecting the tumor-dormant state. The peritoneal macrophages were removed from ear punch-coded tumor-dormant mice by the PPL technique and were assayed for suppression of the CTL response in MLTC cultures. Each mouse was then observed over a 60-day period for development of ascitic tumors. We restricted this study to clinically normal tumor-dormant mice which had <5 x 10^4 L5178Y cells in the peritoneal cavity at the time of the PPL. We evaluated 22 tumor-dormant mice and found that 7 had
Suppressor Macrophages in Tumor-dormant Mice

Table 3
Lack of suppressive activity of AD PC from tumor-dormant mice on cytotoxic T-lymphocyte activity at the effector stage

<table>
<thead>
<tr>
<th>AD PC source</th>
<th>No. of L5178Y cells in peritoneal cavity</th>
<th>% of lysis at the following effector:AD PC:target cell ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor-dormant mice</td>
<td>&lt;10⁶</td>
<td>52.2 ± 3.2</td>
</tr>
<tr>
<td>Tumor-emergent mice</td>
<td>&gt;10⁶</td>
<td>52.2 ± 3.0</td>
</tr>
<tr>
<td>Normal mice</td>
<td>0</td>
<td>58.5 ± 2.7</td>
</tr>
<tr>
<td>None Stimulator cells</td>
<td>51.6 ± 3.9</td>
<td>10:1</td>
</tr>
<tr>
<td>No stimulator cells</td>
<td>6.0 ± 1.2</td>
<td>10:1</td>
</tr>
</tbody>
</table>

- Cytotoxic T-lymphocyte effector cells against L5178Y cells were prepared in a syngeneic MLTC as described in "Materials and Methods." AD PC (10⁵) from tumor-dormant mice, tumor-emergent mice, or normal mice were added to the mixture of 10⁵ Cr-labelled target cells and effector cells at an effector:AD PC:target cell ratio of 100:10:1 or 30:10:1 or 10:10:1 in a final volume of 0.2 ml in microtiter plates.
- Mean ± S.E.
- Stimulator cells were irradiated L5178Y cells added to the MLTC cultures at a responder:stimulator ratio of 100:1.

Macrophage populations which suppressed the CTL response in MLTC cultures and that 15 had nonsuppressive macrophages. The survival curves of these 2 groups of mice, presented in Chart 5, indicate that ascitic tumors developed more rapidly in the group of mice which had suppressive macrophages (p < 0.05, Wilcoxon rank sum test). Thus, the expression of immunosuppressive activity by macrophages from a clinically normal tumor-bearing mouse indicates that the tumor-dormant state will shortly terminate and suggests that induction or activation of macrophages which have increased immunosuppressive activity is a causative factor in tumor emergence. 

DISCUSSION

In this paper, we have focused our attention on the immunosuppressive effects of peritoneal macrophages from tumor-dormant mice and on the relevance of immunosuppression to the termination of the tumor-dormant state. Macrophages are required as accessory cells for a variety of immune responses (15), including the generation of CTL activity. However, normal resident macrophages in amounts 2- to 3-fold greater than those required for accessory cell function can inhibit significantly the in vitro generation of CTL activity (9, 24). Also, relatively small numbers of splenic, peritoneal, or tumor-associated macrophages from tumor-bearing mice can inhibit lymphoproliferative responses (17) and the generation of CTL responses in MLTC cultures (7, 8). In our experiments, peritoneal macrophages from normal DBA/2 mice produced dose-dependent immunoregulatory effects on CTL generation in MLTC cultures. However, by selecting a macrophage dose (3%) in the MLTC cultures at which macrophage populations from only 29% of normal mice were suppressive (Chart 2), we were able to demonstrate a significantly greater incidence of suppressor macrophage activity in tumor-dormant mice.

We observed a direct correlation between the incidence, in tumor-dormant mice, of peritoneal macrophage-mediated immunosuppressive activity and the i.p. tumor burden and showed that most tumor-dormant mice with tumor burdens >10⁴ cells also had suppressor macrophages. The increased immunosuppressive activity in tumor-dormant mice with high tumor burdens could have been due to either an increased number of or increased activity of suppressor macrophages. This observation in itself does not distinguish between the increased suppressor macrophage activity contributes to tumor cell proliferation or is induced as a result of tumor cell proliferation, or a combination of both. However, we also observed that tumor-dormant mice which contain macrophages with increased suppressor activity develop ascitic tumors earlier than do tumor-dormant mice whose macrophages were nonsuppressive. This is strong presumptive evidence that macrophage-mediated suppression of CTL stimulation is a contributing factor in the termination of the tumor-dormant state and development of ascitic tumors. Recent experiments have revealed that adoptive transfer of AD PC from tumor-dormant mice with large tumor burdens to tumor-dormant mice with small tumor burdens results in the early development of ascitic tumors in the recipient mice.

Our previous studies on macrophages have revealed that tumor-dormant mice with small tumor burdens (<10⁴ L5178Y cells) contain macrophages which, when tested at a high effector:target ratio, exhibit no cytolytic activity (21, 22) but which

T. Okayasu and E. F. Wheelock, unpublished observations.
can combine with T-lymphocytes to produce synergistic cytolytic activity (22). In contrast, all tumor-dormant mice with large tumor burdens (>10^6 L5178Y cells) contain macrophages which exhibit cytolytic activity against L5178Y cells when tested at a high effector:target ratio (21). In the present study, the majority of these tumor-dormant mice with large tumor burdens also contained macrophages which produced immunosuppressive effects in vitro when tested at low macrophage:responder cell ratios in an MLTC. Macrophage-mediated cytolytic activity and macrophage-mediated immunosuppression have been observed in the same macrophage population in other systems (8, 10), although it is not known whether the same or different subpopulations were responsible for these 2 effects. In our experiments, we could not assay for both effector functions from individual tumor-dormant mice, because of the limited number of peritoneal macrophages which could be recovered. The lack of direct macrophage-mediated cytotoxicity shown in Table 3 (at a 0:10:1 effector CTL:AD PC:tumor cell ratio) with AD PC from mice with large tumor burdens would appear to be at variance with our earlier report (21). However, the AD PC:target ratio used in this experiment was 10:1, and the assay duration was 18 hr, conditions that are much less sensitive than those used previously (effector:target ratio, 60:1 to 100:1 and 48-hr cytolytic assay).

The majority of studies of immunosuppressive cells in tumor-bearing mice have evaluated their effects in vitro (14). Only a few studies, and none with regard to suppressor macrophages, have evaluated the influence of these cells on tumor progression in vivo. Suppressor T-cells have been implicated in enhanced growth of transplanted tumor cells (4, 5, 18), in induction of UV radiation-induced tumors (3), and in the failure of adoptive T-cell immunotherapy (2). The experiments presented here establish a link between the immunosuppressive effects of macrophages in vitro and tumor progression in vivo.

Our working hypothesis on the mechanisms involved in the maintenance of the tumor-dormant state is that, after peritoneal CTL activity declines to background levels, low-level CTL stimulation continues in the vicinity of proliferating tumor cells. This generated cytolytic activity, in conjunction with cytolytic macrophage activity (21, 22), lyses the tumor cells and prevents their progressive proliferation. Eventually, some event, unidentified as yet, triggers an increase in the immunosuppressive activity of peritoneal macrophages. These macrophages suppress CTL stimulation and permit tumor cells to proliferate without restraint to form an ascitic tumor.

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