Role of Asialo-\text{GM}_{1} \text{ Positive Liver Cells from Athymic Nude or Polyinosinic-Poly-cytidylic Acid-treated Mice in Suppressing Colon-derived Experimental Hepatic Metastasis}

Stefan A. Cohen, Shie-pon Tzung, Ralph J. Doerr, and Martin H. Goldrosen

ABSTRACT

Liver-derived (LD) murine colon adenocarcinoma MCA-38 cells injected into the ileocolic vein (ICV) of C57BL/6 mice developed distinct hepatic foci within 14–21 days and survived for an average of 19–35 days. In contrast, C57BL/6-\text{n}u/\text{nu} mice given injections of LD-MCA cells by the same route did not develop hepatic lesions. Furthermore, \textsuperscript{111}In-labeled LD-MCA-38 tumor cells were rapidly taken up by the liver of conventional mice within 1 h and 73% of the radioactivity remained after 24 h. However, about 60% of the \textsuperscript{111}In-labeled LD-MCA-38 tumor cells were cleared from the liver of nude mice after 24 h. Nonparenchymal liver cells isolated from untreated conventional mice displayed little cytotoxicity against freshly excised \textsuperscript{3}Cr-labeled LD-MCA-38 cells but did lyse the standard natural killer target, YAC-1 tumor cells, in 4 h chromium release assays. On the other hand, nonparenchymal liver cells but not spleen cells from nude mice were cytotoxic to \textsuperscript{3}Cr-labeled LD-MCA-38 \textit{in vitro}. The nonparenchymal liver cell population responsible for tumor killing was phenotypically nonadherent and asialo-\text{GM}_{1} (AsGM\textsubscript{i}) positive. C57BL/6 mice treated with polyinosinic-poly-cytidylic acid [poly(IC)] also displayed cytotoxic activity against LD-MCA-38 tumor cells \textit{in vitro}. Furthermore, poly(IC) treatment of mice 1–8 days after tumor inoculation suppressed the number of hepatic foci and also significantly increased the life span of tumor-bearing mice. Treatment of athymic nude mice or poly(IC)-treated conventional mice with anti-AsGM\textsubscript{i} induced significant numbers of foci and significantly decreased the life span of MCA-38-bearing mice suggesting that AsGM\textsubscript{i}-positive cells in the liver of these mice may inhibit tumor growth \textit{in vivo}. In conclusion, the host defense system of the liver from athymic nude or poly(IC)-treated mice possess AsGM\textsubscript{i}-positive cells that can suppress tumor implantation or tumor growth in the early stages of metastasis in liver.

INTRODUCTION

Hepatic metastasis is the most common cause of death from colon cancer (1, 2). About 70% of patients with advanced colon tumors develop hepatic metastasis (3, 4). After the invasion of the tumor through the portal blood to the liver, there is a progression of colon-derived metastasis from hepatic to extrahepatic sites (5). Despite the concomitant development of extrahepatic cancer, the extent of tumor within the liver determines the length of survival of most patients. Unfortunately, the prognosis for this disease has not changed over the last few decades. Little is known about the role of the host defense system of the liver in controlling tumor invasion. To study this problem with the goal of creating new therapies, more meaningful experimental models need to be generated that more closely simulate the human condition. Several syngeneic (6–8) and xenogeneic (9, 10) systems of colon-derived hepatic metastasis as well as prolonging the life span of mice. In \textit{in vitro}, these cells also display cytostatic and cytotoxic activity against LD-MCA-38 cells.

MATERIALS AND METHODS

Mice. Adult 6- to 10-week-old male C57BL/6J mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Athymic \text{n}u/\text{n}u mice were obtained from Taconic Farms, Germantown, NY. C57BL/6-\text{n}u/\text{n}u + and C57BL/6-\text{n}u/\text{n}u were bred in animal facilities at Roswell Park Memorial Institute (Buffalo, NY). All mice were maintained on a standard mouse diet of pellets and water \textit{ad libitum}.

Reagents. Poly(IC) was obtained from Sigma Chemical Co. (St. Louis, MO). Mice were given i.p. injections of 200 \textmu g of poly(IC) as indicated.

Preparation of LD-MCA-38 Tumor Cells (11). Mice bearing liver tumors between the 3rd and 5th weeks post-ICV injection were sacrificed and pinned to a dissecting board. The liver was exteriorized by a midline incision, and a lobe-by-lobe hepatectomy was performed into a glass Petri dish containing 10 ml 0.25% trypsin-EDTA maintained at 4°C.

Tumor nodules were carefully dissected from normal liver tissue, and nonnecrotic portions were cut into 2–3-mm fragments by a scalpel blade and fine forceps. These were collected into a 50-ml plastic tube containing 10 ml fresh 0.25% trypsin-EDTA and rotated for 15 min at room temperature. The resulting tumor enzyme suspension was diluted

1 The abbreviations used are: MCA-38, murine colon adenocarcinoma 38; LD, liver-derived; CD, culture-derived; NK, natural killer cell; NPC, nonparenchymal liver cells; ICV, ileocolic vein; AsGM\textsubscript{i}, asialo-\text{GM}_{1}; IFN, interferon; poly(IC), polyinosinic-poly-cytidylic acid; HBSS, Hank’s balanced salt solution; Ad-NPC, adherent nonparenchymal liver cells; RS, rabbit serum.
SUPPRESSION OF HEPATIC METASTASIS BY AsGMı* LIVER CELLS

up to 50 ml with RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum plus antibiotics, filtered through a four-layer No. 200 nylon mesh, and spun at 200 × g. The pellet was washed once in tissue culture medium and resuspended to a final concentration of 1.5 × 10^6 cells/ml. The viability of injected cells as determined by trypan blue dye exclusion was between 90 and 95%.

ICV Injection (11). Mice 8–10 weeks old were anesthetized by i.p. injection of 55 mg pentobarbital/kg. A midline incision was made and the ICV was exposed; 1.5 × 10^5 tumor cells in 0.1 ml were injected into the vein with a 30-gauge needle attached to a 1-ml tuberculin syringe, and a cotton-tipped applicator was placed over the injection site for 1 min to prevent excessive bleeding. After the cecum was returned to its proper position in the abdomen, the peritoneal membrane was closed with the use of a 5-0 chromic suture (Ethicon), and the skin was closed with stainless steel clips.

Laparotomy. Two to 3 weeks after ICV injection, mice were reanesthetized by i.p. injection of pentobarbital (50 mg/kg), and a 3-cm midline incision was made from the tip of the sternum to the pubis. All six lobes of the liver were carefully examined anteriorly and posteriorly for tumors by a small stainless steel retractor and saline-moistened cotton-tipped applicator. The numbers of visible liver foci were recorded for each lobe, and the abdomen was closed by a 5-0 Ethylene suture (Ethicon) and stainless steel clips. Hepatic foci greater than 400 could not be accurately counted and were recorded as 400.

Survival. All experimental animals were palpated once per week beginning at the 2nd week until death to assess hepatic tumor growth. The day of death was recorded.

Preparation of NPC. NPC were isolated by modifying a previously described method (14, 15, 18). Briefly, mice were sacrificed by cervical dislocation, the skin over the abdomen and chest was reflected, and the inferior vena cava was ligated above the diaphragm. The inferior vena cava below the liver was cannulated with a heparinized 20-gauge needle, through which the liver was perfused at 37°C with 40 ml of HBSS without Ca^2+ and Mg^2+ (pH 7.2), delivered over 2 min with the outflow through an incision in the portal vein. The liver was then perfused with 100 ml of sterile HBSS (pH 7.6) supplemented with 0.05% collagenase, delivered over 5 min. The distended and blanched liver was removed, rinsed with HBSS, teased apart with a rubber policeman, and passed through a 100-μm nylon mesh screen. Five ml of a single cell suspension were mixed with 7 ml of 30% w/v Metrizamide (Accurate and Scientific Co., Hicksville, NY), dissolved in Guy's balanced salt solution (without NaCl), then gently overlaid with 1 ml of Guy's balanced salt solution (with NaCl), and centrifuged at 550 x g for 15 min at 23°C. NPC were collected from the interface, washed twice in HBSS, and resuspended in media. NPC obtained by this method were >95% viable (as assessed by trypan blue exclusion) and contained less than 3% hepatocytes. Approximately 1 × 10^7 NPC were recovered per liver.

Isolation of NPC Adherent to Plastic (Kupffer Cells). Various numbers of NPC were seeded into microwells of 96-well flat bottomed tissue culture plates and allowed to adhere for 3 h at 37°C in tissue culture medium (containing 20% fetal bovine serum). After incubation, the nonadherent cells were removed and the plates were washed 4 times with warm medium. Greater than 90% of adherent cells were liver macrophages or Kupffer cells as shown by uptake of latex beads and nonspecific esterase staining.

In Vivo Treatment of Mice with Anti-AsGMı. Rabbit anti-AsGMı was purchased from Wako Chemical Co. (Dallas, TX). Mice were given 25-μl injections of anti-AsGMı i.p., 1 day prior to and 4 days after tumor inoculation. Rabbit anti-AsGMı reacts with murine NK cells, fetal liver cells, and mouse monocytcs (19–22).

Assay of Cytotoxic Activity. Target cells were labeled with Na_2^{31}CrO_4 for 1 h at 37°C (100 μCi/10^6 cells). The ^31Cr release assay was performed in 96-well flat bottomed tissue culture plates (Costar, Cambridge, MA). NPC were mixed with 2 × 10^5 tumor target cells in triplicate in a total volume of 200 μl and incubated for 4 h in a humidified atmosphere of 5% CO_2 in air. Supernatants were harvested and counted in a Packard gamma counter. The results were expressed as the percentage of specific release calculated as

\[
\%\text{ inhibition} = 1 - \frac{cpm (NPC + target cells)}{cpm (NPC) + cpm (target cells)} \times 100
\]

Table 1 Induction of experimental hepatic metastasis in C57BL/6, C57BL/6-nu/+, and C57BL/6-nu/nu mice after ICV injection of LD-MCA-38 cells

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. of mice</th>
<th>Mean</th>
<th>Median</th>
<th>Range</th>
<th>P*</th>
<th>% colonization</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6</td>
<td>5</td>
<td>300</td>
<td>400</td>
<td>105–400</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>C57BL/6-nu/+</td>
<td>10</td>
<td>400</td>
<td>400</td>
<td>400</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>C57BL/6-nu/nu</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.001</td>
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* Wilcoxon rank sum test; compared to nu/+ mice.

RESULTS

Colonization of LD-MCA-38 Tumor Cells after ICV Injection into C57BL/6, C57BL/6-nu/+, and C57BL/6-nu/nu. LD-MCA-38 tumor cells were injected into the ICV of C57BL/6 or C57BL/6-nu/+ mice and produced distinct hepatic foci that could be easily quantitated. The hepatic foci formed by this procedure are microscopically apparent 11 days after injection and are visible to the eye 14–21 days after injection. In a typical experiment, up to a maximum of 400 foci/liver were enumerated 21 days after inoculation of LD-MCA-38 cells (Table 1). C57BL/6-nu/+ mice with 400 hepatic foci on day 21 survived an average of 27 days (Fig. 1). Furthermore, 21 days after tumor inoculation into untreated C57BL/6 mice, the liver was the only organ in which A is cpm of experimental, B is cpm from spontaneous release, and C is cpm from target cells. The target cells used in this study were CD-MCA-38 and YAC-1 maintained in tissue culture medium and free of Mycoplasma and LD-MCA-38.

Assay of Cytotoxic Activity. The cytosis assay was performed in 96-well flat bottomed tissue culture plates as described previously (23). From 4 to 8 × 10^4 NPC were mixed with 2 × 10^6 cold tumor target cells in triplicate in a total volume of 200 μl and incubated for 72 h in a humidified atmosphere of 5% CO_2 in air. Wells were pulsed with 0.5 μCi of thymidine for 18 h, the cells were harvested with a semiautomatic cell harvester (Skatron Model 1701), and the cpm were determined by liquid scintillation counting. The percentage of inhibition was determined as

\[
\%\text{ inhibition} = 1 - \frac{cpm (NPC + target cells)}{cpm (NPC) + cpm (target cells)} \times 100
\]
Cytotoxicity and Cytostasis of NPC and Spleen Cells from C57BL/6, Poly(IC)-treated C57BL/6, C57BL/6-nu/+, and C57BL/6-nu/nu Mice against Freshly Excised LD-MCA-38 and YAC-1 Tumor Cells. Previously, this laboratory and others (13-17) have demonstrated that NPC from untreated mice and rats have NK activity against \(^{51}\text{Cr}\)-YAC-1 tumor cells and rats have NK activity against \(^{51}\text{Cr}\)-YAC-1 tumor cells in a 4-h assay. Therefore, the cytotoxicity of NPC from C57BL/6, C57BL/6-nu/+, and C57BL/6-nu/nu mice against freshly excised \(^{51}\text{Cr}\)-LD-MCA-38 was compared with that against YAC-1 cells. As shown in Table 3, Experiment A, NPC from C57BL/6 mice were cytotoxic against YAC-1 tumor cells but not LD-MCA-38 tumor cells. As shown by this laboratory previously (14), treatment of mice with anti-AsGM1, an antibody that inhibits NK activity (20), abolished the ability of NPC to lyse YAC-1 tumor cells. NPC from nude mice did not display cytotoxic activity against \(^{51}\text{Cr}\)-YAC-1 compared with conventional mice (Table 3, Experiment B). Ad-NPC (>90% Kupffer cells) did not exert cytotoxicity against YAC-1 or LD-MCA-38 tumor cells. Interestingly, spleen cells from nude mice did not display cytotoxic activity against LD-MCA-38 but did display significant lysis against YAC-1 tumor cells. NPC from athymic nude nu/nu mice also lysed LD-MCA-38 and YAC-1 tumor cells (Table 3, Experiment C). Poly(IC) which augments NK activity in the liver and spleen (25) and induces serum interferon activity (26)
was injected into C57BL/6 mice and 1 day later the cytotoxicity against both tumors was evaluated. NPC from poly(IC)-treated mice induced significant activity against 51Cr-LD-MCA-38 tumor cells and displayed augmented cytotoxic activity against 51Cr-YAC-1 compared with untreated mice (Table 3, Experiment D).

Because no cytotoxic activity by NPC from conventional mice against LD-MCA-38 cells was detected using the short-term 4-h 51Cr release assay, a longer term 72-h cytostatic 3H-thymidine uptake assay was used. As illustrated in Table 4, NPC from untreated conventional mice from 4 separate experiments exerted 30-50% cytostatic activity against LD-MCA-38 cells. This activity was not detected with Ad-NPC (>90% Kupffer cells). In contrast, NPC from nude mice exerted augmented cytostatic activity (95-97%) against LD-MCA-38 tumor cells. Furthermore, treatment of nude mice with anti-AsGM1 totally reversed the cytostatic activity of NPC in one experiment (Table 4, Experiment B) and partially reversed in another experiment (Table 4, Experiment C). Poly(IC) given 1 day prior to sacrifice also boosted the cytostatic activity of NPC against LD-MCA-38 tumor cells (Table 4, Experiments D and E).

**In Vivo Effect of Anti-AsGM1 on the Number of Hepatic Foci and Survival of LD-MCA-38 and CD-MCA-38 Tumor Cells in Athymic nu/nu Mice and Poly(IC)-treated C57BL/6 Mice.** Previously, we have shown (27) that treatment of C57BL/6 mice with anti-AsGM1 1 day prior to and 3 days after LD-MCA-38 tumor inoculation reduced survival from 35 to 17 days (P < 0.0005). Since anti-AsGM1 treatment of nude mice caused a decrease in the cytotoxic activity of NPC against YAC-1 cells and LD-MCA-38 cells and cytostatic activity of NPC against LD-MCA-38 tumor cells (Tables 3 and 4), the effect of this treatment on the number of hepatic foci and survival of nude mice given LD-MCA-38 tumor cells was determined. As shown in Table 5, athymic nu/nu mice developed only a median of 2 foci/liver and as illustrated in Fig. 2 survived an average of 60 days. In parallel experiments, when these mice were given anti-AsGM1 followed by ICV injection of LD-MCA-38 cells, they developed dramatically more hepatic foci (400) and their survival time was significantly decreased to an average of only 19 day (P < 0.0005). C57BL/6 mice inoculated with CD-MCA-38 cells by the ICV route did not develop any hepatic foci (day 21) and prior treatment of mice with anti-AsGM1 did not induce colonization or death (data not shown). BALB/c-nc/nu/nu mice were also able to resist hepatic metastasis and 80% did not have hepatic foci on day 21 (Table 4) and survived for at least 80 days.

The effect of poly(IC) on the hepatic metastasis and survival of tumor bearing mice was also determined. As illustrated in Table 5, poly(IC) given to C57BL/6 mice 1 day after LD-MCA-38 tumor cell administration dramatically inhibited the number of hepatic foci on day 14 (median, 400 versus 1). This treatment also increased their mean life span from 20 to 70 days (P < 0.0005) (Fig. 3). The administration of anti-AsGM1 before and after tumor inoculation and poly(IC) treatment resulted in a significant increase in the number of hepatic foci (1 versus 400) as well as a decrease in mean survival from >70 to 24 days (Fig. 3). Poly(IC) also suppressed tumor progression and in-
creased life span if given 8 days after tumor inoculation but lost its effect if given 14 days after tumor inoculation (Table 6).

DISCUSSION

In the present study, we correlated the ability of the liver and its subpopulations from immunocompetent, immunostimulated, and congenitally immunodeficient mice to resist colon-derived tumor colonization after ICV administration of LD-MCA-38 tumor cells and lyse or inhibit colon tumor growth in vitro. We demonstrated that (a) NPC from immunocompetent C57BL/6 mice although containing AsGmi-positive liver cells had little or no cytotoxic activity but did exert cytostatic activity against freshly excised LD-MCA-38 in vitro; (b) AsGmi-positive liver cells from athymic nude mice were cytotoxic and exhibited augmented cytostatic activity against LD-MCA-38 in vitro and suppressed the colonization of the liver by LD-MCA-38 cells; (c) AsGmi-positive liver cells from poly(IC)-treated C57BL/6 mice also were cytotoxic and exhibited augmented cytostatic activity against LD-MCA-38 in vitro and inhibited the invasion of LD-MCA-38 tumor cells into the liver.

Spontaneous metastasis initially involves local invasion from the primary neoplasm and penetration of the tumor cells into the lumen of blood vessels (28, 29). However, there are few murine tumors that spontaneously metastasize to the liver. Those that do are reticuloendothelial sarcomas such as M5076 and RCS2 which are histologically different from colon tumors (8, 30). Transplantable mouse colon tumors such as MCA-38 and RCS2 which are histologically different from colon tumors had little or no cytotoxic activity but did exert cytostatic activity against freshly excised LD-MCA-38 in vitro; (b) AsGmi-positive liver cells from athymic nude mice were cytotoxic and exhibited augmented cytostatic activity against LD-MCA-38 in vitro and suppressed the colonization of the liver by LD-MCA-38 cells; (c) AsGmi-positive liver cells from poly(IC)-treated C57BL/6 mice also were cytotoxic and exhibited augmented cytostatic activity against LD-MCA-38 in vitro and inhibited the invasion of LD-MCA-38 tumor cells into the liver.

In our previous studies (11), we demonstrated that LD-MCA-38 tumor cells injected into the ICV of conventional mice colonized the liver in a stepwise and reproducible fashion. This tumor colonization resulted in colonization of the liver (31). However, this tumor...
formed a diffuse pattern of metastasis in the liver which could not be easily quantitated.

Our previous results (27) have shown that untreated C57BL/6 mice do mount an antimitastatic response in vivo against LD-MCA-38 tumor since anti-AsGMi treatment significantly reduced their life span from 35 to 17 days. In addition, NPC from C57BL/6 mice exerted cytostatic but not cytotoxic activity against LD-MCA-38 cells in vitro. However, NPC from these mice can lyse the standard NK target YAC-1 and lysis could be abrogated by prior treatment with anti-AsGMi (14, 20). Itoh et al. (17) demonstrated using immunofluorescence that nonadherent NPC from C57BL/6 mice contained 18% AsGMi-positive lymphocytes. Thus, resident AsGMi-positive cells present in the livers of conventional C57BL/6 mice do not exert a direct cytotoxic effect on freshly excised tumor cells in short term 4-h assays. Other studies have also shown that freshly excised tumor cells are resistant to natural cytotoxic activity (32, 33). The resident cytostatic activity exhibited by AsGMi NPC from conventional mice in vitro may not prevent tumor implantation or colonization of the liver but may instead contribute to suppression of tumor growth. However, the cytostatic activity displayed by NPC does not appear to be due to conventional NK cells since NPC from NK-deficient beige mice were as cytostatic against LD-MCA-38 as those from their conventional littermates. Furthermore, no difference was seen in the survival of beige mice compared to their matched littermates in response to ICV-administered LD-MCA-38 cells (data not shown). It is known that AsGMi cells exist in the livers of mice devoid of NK activity (21, 22).

Wiltrout et al. (34) have shown that anti-AsGMi treatment of C57BL/6 mice exhibited increased formation of experimental metastasis in the lung and induced metastasis in the liver after i.v. challenge with B16 melanoma. This increased metastasis coincided with decreased blood, spleen, and liver NK (YAC-1) activity. In contrast to LD-MCA-38 tumor cells, B16 tumor cells are from a cultured cell line which is usually sensitive to NK cytotoxicity. Since cultured tumor cells tend to drift from their original phenotype (35), LD-MCA-38 may be a more relevant tumor to study. Furthermore, LD-MCA-38 tumor cells grown in culture (CD-MCA-38 cells) do not produce hepatic colonies following ICV injection. This inability of CD-MCA-38 cells to colonize the liver is not the result of susceptibility to AsGMi-positive cells since the abrogation of in vivo AsGMi activity did not result in colonization of the liver (data not shown). The reason for the lack of colonization by CD-MCA-38 cells may be more dependent on other properties of the tumor such as their decreased motility rather than an increase in susceptibility to immune attack (36).

Nude mice have a significantly greater capacity to resist tumor colonization than untreated conventional mice. The liver from nude mice may more efficiently suppress or prevent the tumor from initially seeding the liver. In this regard, the liver of C57BL/6-nu/nu mice can clear 111In-LD-MCA-38 cells at a faster rate than the liver of C57BL/6-nu/+ . Furthermore, when nude mice were pretreated with anti-AsGMi antibody, these mice survived about the same length of time as anti-AsGMi-treated conventional mice (19.5 versus 17 days, respectively). Since the cytotoxic and cytostatic activity in the liver of nude mice reappeared 7 days after cessation of antibody treatment (data not shown), this suggests that AsGMi-positive cells from nude mice inhibit tumor implantation or early tumor growth but not established tumors.

There are many reports that demonstrate that NK cells from nude mice have greater cytotoxicity than from conventional mice (37, 38). However, more efficient cytotoxicity may not be entirely responsible for the antimetastatic effect since splenic NK cells from nude mice which have greater cytotoxicity against YAC-1 cells than spleen from conventional mice still do not lyse freshly excised LD-MCA-38 cells. It is likely the microenvironment of liver from nude mice may be different from the spleen. In this regard, NPC from nude mice produced detectable IFN-α/β (20-40 units/ml) in culture while spleen cells do not (data not shown). We speculate that hepatic NK cells from nude mice are activated in situ in part by local production of IFN-α/β which broadens their specificity and directly lyses LD-MCA-38 cells. This is consistent with the fact that freshly excised tumors have been shown to be susceptible to IFN-activated NK cells (32, 33).

The interferon inducer, poly(IC), injected into C57BL/6 mice induced cytotoxicity and augmented cytostasis against LD-MCA-38 tumor cells in vitro. Furthermore, the number of hepatic foci in the liver of poly(IC)-treated mice was significantly reduced when measured 14 days after tumor inoculation. When mice were given anti-AsGMi prior to poly(IC), then the number of hepatic foci increased to those of untreated mice. Poly(IC) treatment of mice bearing covert or micrometastasis (day 8) was also effective (Table 6). In contrast, poly(IC) had no effect if administered to mice bearing visible hepatic tumors (day 14). AsGMi-positive cells have been shown to contribute to the therapeutic efficacy of IL-2 against MCA sarcoma soon after implantation. However, once tumors were established, AsGMi-positive cells had no detectable role in mediating tumor regression (39). As in the case with nude mice, AsGMi-positive cells from poly(IC)-treated mice may influence tumor implantation but have little or no influence on established tumors.

Kupffer cells did not exhibit antitumor activity in vivo. Administration of NPC from nude mice which consist of greater than 90% Kupffer cells did not lyse or prevent the proliferation of LD-MCA-38 cells (Tables 4 and 5). Furthermore, the administration of anti-AsGMi into mice or treatment of NPC with anti-AsGMi plus complement does not abolish the number of Kupffer cells or their phagocytic activity (14, 17). Anti-AsGMi also does not inhibit the tumoricidal activity of macrophages (40). AsGMi treatment of nude mice led to increased numbers of hepatic foci but no extrahepatic metastases suggesting that other mechanisms may operate to prevent the release of tumor from the liver.

In conclusion, the experiments in this paper indicate that AsGMi-positive cells in the liver from conventional mice do not prevent tumor implantation but have the capacity to slow focal tumor growth. In contrast, AsGMi-positive cells in the liver from nude or poly(IC)-treated mice which differ from conventional AsGMi-positive liver cells can suppress tumor implantation or early tumor growth.

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REFERENCES

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