Correlation between Sering Antitumor Activity and Concomitant Resistance in Mice Bearing Nonimmunogenic Tumors

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ABSTRACT

Serum from mice bearing five weakly immunogenic or nonimmunogenic tumors inducing concomitant resistance exhibited a growth-inhibitory activity on in vitro proliferation of the tumor cells. This activity was proportional to the intensity of concomitant resistance and correlated with the capacity to restrain metastatic development. It was not attributable to cytotoxic antibodies, was relatively nonspecific, and operated through a cytostatic and reversible mechanism. All attempts to transfer antitumor resistance in vivo by serum inoculation have failed, but this could be attained by parabiosis. Physical and chemical serum treatments suggest that heat-, acid-, and alkali-resistant peptide(s) with molecular weights ranging from 1000 to 3000 could account for this inhibitory effect.

INTRODUCTION

Concomitant resistance is the phenomenon according to which in a tumor-bearing host the growth of secondary implants of the same tumor at a distant site is inhibited. It was described many years ago (1) and it can be induced by both immunogenic and nonimmunogenic tumors (2, 3). Several authors have demonstrated that classical immunological mechanisms, mainly mediated by T-cells and macrophages, could explain, at least in part, the concomitant resistance generated by strongly immunogenic tumors (4-6). However, for weakly immunogenic and nonimmunogenic tumors, the nature of this phenomenon remains largely unknown; it has been detected in euthymic, nude, and macrophage-depleted mice, suggesting that T-cells and macrophages do not play a significant role (7-9).

In previous papers (9, 10) we have shown that concomitant resistance induced by several nonimmunogenic murine tumors operated through a cytostatic mechanism and without the local participation of host cells. In effect, in the secondary tumor implant undergoing concomitant resistance, histological examination revealed the presence of well preserved tumor cells without any sign of necrosis and without any host cell infiltration, contrasting with conventional immunological rejection. Furthermore, splenic and lymph node cells from mice undergoing concomitant resistance were not cytotoxic for the tumor cells, either in vivo or in vitro.

In this paper, we have explored humoral mechanisms in an attempt to explain the concomitant resistance induced by five weakly immunogenic or nonimmunogenic murine tumors. We found that serum from tumor-bearing mice lacked detectable cytotoxic antibodies but exhibited an in vitro growth-inhibitory activity on the tumor cells, which was proportional to the size of the primary tumor and to the intensity of concomitant resistance.

MATERIALS AND METHODS

Mice. BALB/c and AKR mice of both sexes and 2-4 months old were used throughout. They were raised in our colony and maintained on Cargill pellets and water ad libitum.

Nude BALB/c mice were obtained from the Comisión Nacional de Energía Atómica, Argentina, and kept under relatively aseptic conditions.

Parabiotic BALB/c mice were prepared by joining pairs of BALB/c mice, 2-3 months old, 20-25 g, in parabiotic union involving the skin, underlying panniculus, and peritoneal cavities; cross-circulation is established around day 7 according to our previous experience. Animals were age and sex matched within each experiment.

Serum. Normal or tumor-bearing mice were bled through the retroorbital plexus. The blood was kept at room temperature for 1 h for clotting; serum obtained by centrifugation was stored at −20°C until used. Whenever necessary it was sterilized through a 0.45-μm filter.

Tumors. LB (BALB/c origin) is a nonimmunogenic T-lymphoid leukemia which induces strong concomitant resistance (9, 11). The TD50 was 10⁴ cells along the different passages.

L15 (AKR origin) is a nonimmunogenic lymphoid leukemia inducing concomitant resistance; TD50 was calculated as 6 × 10⁴ cells.

CM (BALB/c origin) is a nonimmunogenic mammary adenocarcinoma inducing concomitant resistance (9). TD50 was calculated as 10⁵ cells.

M1 (BALB/c origin) is a nonmetastatic and weakly immunogenic mammary adenocarcinoma which induces an early and strong concomitant resistance (12).

MM2 is a metastasizing mammary adenocarcinoma derived from M1 as described previously (12, 13). It shows the same weak immunogenicity as the parental M1 tumor but induces a late and weaker concomitant resistance (12).

Concomitant Resistance Assay. Mice received a s.c. tumor implant in the right flank followed at different intervals by a second s.c. implant of the same tumor in the left flank. The controls were challenged only in the left flank. The titer of concomitant resistance was defined as the ratio between TD50 of the second challenge in tumor-bearing mice and TD50 in control mice and expressed as a function of the median time between the day of the second challenge and the day this implant became palpable in the controls. Tumor volume was calculated according to the method of Attia and Weiss (14): Volume = 0.4(ab²)

Received 1/18/90; accepted 7/30/90.

1 This study was supported by national grants from CONICET, Fundaleu, Fundación Antorchas, and Fundación Alberto J. Roemmers.
2 Member of Research Career, CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas).
3 Fellow of Fundaleu (Fundación para Combatir la Leucemia).

5 The abbreviations used are: TD50 number of tumor cells able to grow s.c. and kill 50% of the mice; GIU50, reciprocal of the serum dilution producing 50% inhibition of [3H]thymidine as compared with medium only; CU50, reciprocal of the serum dilution producing 50% of specific lysis.

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body (glass cylinder) 6 months after its s.c. implantation in a BALB/c mouse of our colony; and S180, murine sarcoma maintained in ascitic form by serial i.p. passages in Swiss mice.

Medium. The medium used throughout was RPMI 1640 (Gibco, Grand Island, NY) supplemented with 10% fetal calf serum, 100 μg/ml penicillin, and 150 μg/ml streptomycin.

[^H]Thymidine Uptake Assay. Proliferation of normal and tumor cells in 0.1 ml of medium was determined in 96-well microtiter plates (NUNC, Denmark) in the presence of 0.1 ml of several 2-fold dilutions of serum from normal or tumor-bearing mice. Immediately afterwards, the cultures were pulsed with[^H]thymidine (Dupont, NEN Research Products, Boston, MA) at a final concentration of 1 μCi/ml and the mixture was incubated at 37°C for 18-24 h in 5% CO2 and harvested with an automated cell harvester. The radioactivity incorporated into the cells was counted in a liquid scintillation Beta counter (Beckman). The assays were usually carried out in quadruplicate or sextuplicate and the percentage of inhibition for each serum dilution was determined as

\[
\% \text{ of inhibition} = \left(1 - \frac{cpm \text{ of serum} - \text{background}}{cpm \text{ of control} - \text{background}}\right) \times 100
\]

The titer of growth-inhibitory activity was defined as the reciprocal of the serum dilution producing 50% inhibition of[^H]thymidine uptake by target cells as compared with medium only and was expressed as GIU50/ml.

Complement-dependent Cytotoxic Assay. This test was carried out according to the method of Rao et al. (15). The titer of cytotoxic antibodies against tumor cells labeled with 51Cr (Dupont) was defined as the reciprocal of the serum dilution producing 50% of specific lysis and expressed as CU50/ml. Radioactivity was measured in a Gamma counter (Beckman).

Serum Fractionation. Serum from normal or tumor-bearing mice was subjected to ultrafiltration through Centricon-30 (30,000 molecular weight cutoff) microconcentrators (Amicon, Danvers, MA). The residues were resuspended at original volume and the filtrates were successively filtered through Centricon-10, Centricon-3, and Diaflow YM2 (1000 molecular weight cutoff).

Physical, Chemical, and Enzymatic Serum Treatments. Serum from normal and tumor-bearing mice was: (a) heated at 56°C for 30 min or at 100°C for 3-10 min; (b) acidified at pH 4 with 1 N HCl or alkalized at pH 11 with 1 N NaOH for 3 h at 4°C, with controls receiving 0.5 N HCl plus 0.5 N NaOH at the beginning and end of the 3-h incubation; (c) dialyzed and aliquots (0.2 ml) of this dialyzable serum fraction were incubated with 0.6 ml of different enzymes for 1 h at 37°C; the mixtures were then boiled for 5 min to inactivate the enzymes according to the method of Schiffman et al. (16); controls were aliquots of boiled serum; in effect, fluid collected by sterile sponges placed near the primary tumor or the secondary implant exhibited a growth-inhibitory activity comparable to that found in serum and significantly higher than that attained in sponges placed in normal mice (data not shown).

RESULTS

LB Growth-inhibitory Activity in the Serum of LB-bearing Mice

Growth of LB tumor, initiated with a s.c. inoculum of 10⁶ LB cells, is plotted in Fig. 1a. Fig. 1b shows the kinetics of concomitant resistance against a secondary s.c. challenge of LB cells; its titer increased as a function of LB tumor size. Serum from 207 BALB/c mice bearing a s.c. LB tumor during 5, 11, 14, 16, and 18 days exhibited, in a[^H]thymidine uptake assay, a titer of LB growth-inhibitory activity which was 1, 2.2, 6.3, 8.9, and 18.2 times greater than that observed with normal serum (mean of 14 experiments). No significant difference was seen between LB-bearing mice which have been challenged (n = 83) or not (n = 124) with a secondary LB implant not growing because of concomitant resistance (Fig. 1c). This indicates that this antiproliferative effect was a function of the primary tumor size and independent of the presence of a secondary implant; individual values taken from one experiment chosen as example are shown in Table 1. Fig. 1c also shows that the same growth-inhibitory activity was demonstrable in the serum of 9 nude BALB/c mice bearing an LB tumor; this correlates with the fact that these mice exhibit the same concomitant resistance against LB as do euthymic mice (9, 10). The LB growth-inhibitory activity was confirmed using a nonisotopic assay of LB proliferation by counting viable cells with the trypan blue exclusion method: the growth of 0.3-1×10⁶ LB cells/ml cultured in 25-cm² tissue culture flasks was inhibited up to 92.1 ± 7.9% (day 3 of culture, mean ± SE of 2 experiments) and 96.4 ± 3.6% (day 5) in the presence of 10% of serum from mice bearing LB for 17 days, as compared with normal serum; cell death was minimal and similar in both groups. It is noteworthy that the same growth-inhibitory activity was observed in the serum of mice bearing LB in ascitic form and also inducing concomitant resistance. In addition, growth-inhibitory activity of LB-bearing mice was not restrained to serum; in effect, fluid collected by sterile sponges placed near the primary tumor or the secondary implant exhibited a growth-inhibitory activity comparable to that found in serum and significantly higher than that attained in sponges placed in normal mice (data not shown).

L15 Growth-inhibitory Activity in the Serum of L15-bearing Mice

The growth of L15 tumor initiated with a s.c. inoculum of 10⁶ L15 cells and the kinetics of concomitant resistance against a secondary challenge of L15 cells are shown in Fig. 2, a and b.
SERIC ANTITUMOR ACTIVITY IN TUMOR-BEARING MICE

Table 1 Effect of serum from mice bearing LB during 18 days on LB proliferation

<table>
<thead>
<tr>
<th>Final serum dilution</th>
<th>Group 1*</th>
<th>Group 2*</th>
<th>Normal serum</th>
<th>Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/2</td>
<td>ND</td>
<td>282 (1)</td>
<td>3,862 ± 787 (3)</td>
<td></td>
</tr>
<tr>
<td>1/4</td>
<td>300 ± 100 (3)*</td>
<td>490 ± 134 (4)*</td>
<td>5,695 ± 361 (8)</td>
<td></td>
</tr>
<tr>
<td>1/8</td>
<td>989 ± 58 (4)*</td>
<td>856 ± 203 (4)*</td>
<td>5,491 ± 106 (8)</td>
<td></td>
</tr>
<tr>
<td>1/16</td>
<td>1,704 ± 190 (4)*</td>
<td>1,211 ± 100 (7)*</td>
<td>5,968 ± 116 (8)</td>
<td></td>
</tr>
<tr>
<td>1/32</td>
<td>2,667 ± 170 (4)*</td>
<td>2,682 ± 113 (5)*</td>
<td>6,995 ± 91 (5)</td>
<td></td>
</tr>
<tr>
<td>1/64</td>
<td>4,645 ± 150 (6)*</td>
<td>5,070 ± 280 (6)*</td>
<td>8,138 ± 40 (6)</td>
<td></td>
</tr>
<tr>
<td>1/128</td>
<td>8,619 ± 563 (6)</td>
<td>9,288 ± 232 (5)</td>
<td>9,695 ± 436 (6)</td>
<td></td>
</tr>
</tbody>
</table>

P < 0.001 as compared with normal serum.

Fig. 2. (a) Growth of s.c. L15 (O) or CM (■) tumors. L15, mean ± SE (bars) of 5 experiments involving 37 mice; average death latency, 20 ± 1 days. CM, mean ± SE of 6 experiments involving 27 mice; average death latency, 60 ± 3 days. (b) Concomitant resistance induced by L15 (O) or CM (■). L15, mean ± SE of 2 experiments involving 40 mice; CM, mean ± SE of 4 experiments involving 40 mice. (c) Growth-inhibitory activity of serum from L15 (O) or CM (■)-bearing mice as compared with normal serum (——). L15, mean ± SE of 3 experiments involving 20 mice; P < 0.01 (day 12) and P < 0.05 (day 16). CM, one experiment involving 12 mice. (d) Complement-dependent cytotoxic antibodies in serum from L15 (O) or CM (■)-bearing mice. One experiment involving 12 mice for each tumor. ——, normal mice; ———, BALB/c mice which had rejected L15.

Serum from AKR mice bearing a s.c. L15 tumor during 7, 12, and 16 days exhibited, in a [3H]thymidine uptake assay, a titer of L15 growth-inhibitory activity which was 2.5, 4.6, and 13.7 times greater than that observed with normal serum (Fig. 2c).

CM Growth-inhibitory Activity in the Serum of CM-bearing Mice

The growth of CM tumor initiated with a s.c. inoculum of 1 × 10⁶ CM cells and the kinetics of concomitant resistance against a secondary challenge of CM cells are also shown in Fig. 2, a and b; concomitant resistance increased as a function of tumor size but peaked more slowly than that induced by LB and L15. Serum from BALB/c mice bearing a CM tumor during 13, 31, 41, 51, and 57 days exhibited, in a [3H]thymidine uptake assay, a titer of CM growth-inhibitory activity which was 1.7, 1.9, 3.2, 3.9, and 9 times greater than that attained with normal serum. Again, the titer of this growth-inhibitory activity correlated with the intensity of concomitant resistance.

Growth-inhibitory Activity in the Serum of M3 and MM3-bearing Mice

Serum was collected from mice bearing the nonmetastatic M3 and the highly metastatic MM3, when both tumors had reached a similar size (mean volume, 8,000 mm³). At this stage, M3 induces a stronger concomitant resistance against i.v. challenge of M3 and MM3 cells than MM3. Serum was not assayed on M3 or MM3 because they were difficult to culture; instead CM was chosen as target, because of its similar histological pattern. Serum from M3-bearing mice markedly inhibited the proliferation of CM cells in a [3H]thymidine uptake assay as compared with normal serum; serum from MM3-bearing mice was also inhibitory but to a significantly lesser degree (Table 2). These results suggest a correlation between concomitant resistance, absence of metastases, and growth-inhibitory activity in serum.

Search for Antitumor Cytotoxic Antibodies

The titer of cytotoxic antibodies (CU50/ml) in the serum of tumor-bearing and normal mice was determined using a 51Cr release assay and expressed as the ratio between CU50 of tumor-bearing serum and CU50 of normal serum. For LB tumor, the ratio was 1.4 ± 0.5, 0.5 ± 0.2, 0.6 ± 0.2, and 0.9 ± 0.0 corresponding to days 6, 11, 14, and 18 of tumor growth (Fig. 1d). The absence of significant complement-dependent cytotoxicity strongly contrasts with the in vitro growth-inhibitory activity (Fig. 1c; Table 1). Furthermore, serum from AKR mice which had rejected an LB implant (positive control of the 51Cr release assay) had a very high cytotoxic titer against 51Cr-LB cells (ratio related to normal serum, 465.4 ± 49.7) but did not show anti-LB effect when evaluated by the [3H]thymidine uptake assay. As for L15 and CM (Fig. 2d), M3, and MM3 (12), the results were similar to those obtained with LB. These results indicate that the antitumor effect found in the serum of mice bearing these five tumors is not mediated by complement-dependent cytotoxic antibodies.

Attempts to Mimic Concomitant Resistance by LB Serum Transfer

A total of 40 mice challenged with 1–5 × 10⁶ LB cells i.p. or s.c. and receiving 0.2–1.2 ml of LB serum i.p. or s.c. daily,
starting on the day of tumor inoculation, showed the same growth kinetics and incidence of LB tumor as 74 mice receiving normal serum or none. The same negative result was obtained in 12 mice, when LB serum was inoculated every 3–4 h during 12 days. A rapid disappearance of LB growth-inhibitory activity was observed in serum from normal mice inoculated with i.p. or i.v. LB serum; the possibility that an inhibitor of the latter was present can be discarded since in vitro normal serum does not counteract the antiproliferative effect of LB serum (data not shown).

Taking into account the striking difficulty in mimicking concomitant resistance by LB serum inoculation, we tried to transfer LB serum through cross-circulation using parabiotic mice. To this effect, 11 pairs of mice were joined in parabiosis with unchallenged normal mice; 7 days later the titer of growth-inhibitory activity was similar in both partners.

Table 3 Effect on LB proliferation of serum from normal and LB-bearing partners of parabiotic mice

<table>
<thead>
<tr>
<th>Mice</th>
<th>Normal partners</th>
<th>LB-bearing partners</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal single</td>
<td>45.2</td>
<td>438.6</td>
</tr>
<tr>
<td>LB-bearing single</td>
<td>315.6</td>
<td>495.2</td>
</tr>
<tr>
<td>LB-bearing-normal pair 1</td>
<td>712.6</td>
<td>588.9</td>
</tr>
<tr>
<td>LB-bearing-normal pair 3</td>
<td>265.4</td>
<td>402.2</td>
</tr>
<tr>
<td>Ex-parabiotic normal single</td>
<td>40.4</td>
<td>74.7</td>
</tr>
</tbody>
</table>

* Randomized from 3 mice.

Characteristics of the Growth-inhibitory Activity Found in the Serum of Mice Bearing LB for 16–18 Days

Reversibility. LB cells were incubated with normal or LB serum and were divided into two groups. Immediately after receiving the serum the first group was pulsed with [3H]thymidine; after incubating for 18 h, growth inhibition of LB cells by LB serum was 89.4 ± 4% (mean ± SE of three experiments) as compared with normal serum. The second group was not pulsed with [3H]thymidine and after 18 h of incubation, LB cells were washed and incubated for another 18 h with fresh medium and [3H]thymidine. After this period, no LB growth inhibition was observed. This means that the growth-inhibitory activity of LB serum is reversible. This was confirmed using the nonspecific assay. LB cells, 1 × 10⁶/ml, prevented from growing up to 91.9% (day 6 of culture) in the presence of 10% of LB serum were washed and again incubated with fresh medium only; in this condition the LB cells grew as in the controls.

Relative Nonspecificity. The effect of LB serum on the growth of 16 normal or tumor cell lines of different origin was evaluated using the [3H]thymidine uptake assay. Results were expressed as the ratio GIU₅₀ of LB serum/GIU₅₀ of normal serum, considering 100% the rate attained on LB proliferation. All cell lines were inhibited but in a different proportion. As shown in Fig. 3, all lymphoid cells were as inhibited as LB (75–100%). On the other hand, the majority of fibroblast cells were inhibited in a lesser degree (28.9–33.9%) with the exception of S180 (100% of inhibition). As for epithelial cells, the effect of LB serum on Hep-2 and CHO-K1 proliferation was similar to that on LB, but on CM and B16/F10, it was significantly less. The human erythroleukemia K-562 and the murine monocytic P388 showed an intermediate pattern of inhibition (48.5 and 43.4%, respectively) while the human malignant histiocytic U-937 exhibited a low one (24.4%).

Physical and Chemical Properties. Normal and LB sera were subjected to ultracentrifugation through filters of 30,000, 10,000, 3,000, and 1,000 molecular weight cutoff. This yielded five fractions: fraction 1, M₆ > 30,000; fraction 2, 30,000 > M₆ > 10,000; fraction 3, 10,000 > M₆ > 3,000; fraction 4, 3,000 > M₆ > 1,000; and fraction 5, M₆ < 1,000. All fractions were assayed, in two separate experiments, on LB proliferation, using the [3H]thymidine uptake assay. Fractions 1 and 4 of LB serum exhibited a similar growth-inhibitory activity as whole LB serum while the remaining fractions of normal and LB serum were not significantly different from whole normal serum (Table 4). At a second stage, whole LB serum and fraction 1 were subjected to dialysis (12,500 molecular weight cutoff); growth-
Growth-inhibitory activity remained stable when samples were either dialyzed against Jaffé et al.), or uric acid (method of Henry, Sobel, and Kim) if serum did not contain urea (urease method), creatinine (method of effect of trypsin and carboxypeptidase A would indicate that the remaining adherent tumor and normal cells were stimulated with 2.5 μg/ml of phytohemagglutinin for 48 h before adding the serum and [3H]thymidine. Normal human (I x 10^5/well) and murine (5 x 10^5/well) lymphocytes were added (1-3 x 10^5/well) simultaneously with the serum and [3H]-thymidine. Normal human (I x 10^5/well) and murine (5 x 10^5/well) lymphocytes were added (0.2-0.3 x 10^5/well) to the plates 24 h before the serum and [3H]-thymidine. The remaining adherent tumor and normal cells were added (0.2-0.3 x 10^5/well) to the plates 24 h before the serum and [3H]-thymidine.

Tumor cells growing in suspension (LB, L1210, P388, YAC-1, K-562, U-937, and S180) were added (1-3 x 10^5/well) simultaneously with the serum and [3H]-thymidine. The remaining adherent tumor and normal cells were added (0.2-0.3 x 10^5/well) to the plates 24 h before the serum and [3H]-thymidine.

S180) were added (1-3 x 10^5/well) simultaneously with the serum and [3H]-thymidine. Normal human (I x 10^5/well) and murine (5 x 10^5/well) lymphocytes were stimulated with 2.5 μg/ml of phytohemagglutinin for 48 h before adding the serum and [3H]-thymidine. The remaining adherent tumor and normal cells were added (0.2-0.3 x 10^5/well) to the plates 24 h before the serum and [3H]-thymidine.

These results suggest that growth-inhibitory activity is related to heat-stable peptide(s); the lack of effect of trypsin and carboxypeptidase A would indicate that internal basic residues or free carboxyl groups are not necessary for activity, or not available to the enzymes. Additionally, LB serum did not contain urea (urease method), creatinine (method of Jaffé et al.), or uric acid (method of Henry, Sobel, and Kim) in concentrations higher than in normal serum and no interferon activity was detected using the cytopathic effect of vesicular stomatitis virus on murine L-929 cells (data not shown).

**DISCUSSION**

Concomitant resistance to a second tumor challenge has been described for both immunogenic and nonimmunogenic tumors (2, 3). For the former, there is convincing evidence that classical immunological mechanisms could explain, at least in part, the nature of the phenomenon (4-6) but for nonimmunogenic tumors, the mechanisms involved remain speculative. Two non-immunological hypotheses have been formulated: Ehrlich (1) and Tyzzer (17), believed that nutrients essential for tumor growth are consumed by the primary tumor making it difficult for a secondary implant to develop (theory of athrepsis); alternatively, antiproliferative tissue-specific (18) and nonspecific (8, 19, 20) substances produced or induced by the primary tumor could be responsible for the inhibition of secondary tumor implants. Several reports have suggested that nutritional deficiencies might inhibit or retard tumor growth (21-23). On the other hand, mitotic inhibitors have been extracted from tumors and organs of tumor-bearing animals (24) or have been detected in supernatants of cultured tumor cells (25), ascitic fluid (26), or serum from patients or animals bearing a tumor (24, 27-29).

However, to our knowledge, no study has been designed to correlate a systemic nutritional deficiency or a putative inhibitory factor with the induction of concomitant resistance. As for nonimmunogenic tumors, all data concerning effectors were essentially negative, indicating that neither T-cells, macrophages, natural killer, nor other host cells play a main role (7-10). On the other hand, evidence has accumulated indicating that this concomitant resistance is relatively nonspecific and operates through a cytostatic rather than a cytotoxic mechanism (8, 10); both traits contrast with conventional immunological rejection.

In this paper, we have described that serum from mice bearing
5 weakly immunogenic or nonimmunogenic tumors of different histological types exhibited, in vitro, a tumor growth-inhibitory activity which was proportional to the concomitant resistance induced by each tumor and to the capacity to restrain metastatic development. Complement alone or complement-dependent cytotoxic antibodies could not account for this effect; moreover, the fact that tumor-bearing nude mice showed the same pattern of growth-inhibitory activity suggested that T-cells were not involved.

More detailed studies were carried out using the LB model. In this regard, growth-inhibitory activity induced in vitro by LB serum proved to be reversible and relatively nonspecific and was attributable, at least in part, to a heat-, acid-, and alkaliresistant peptide(s) localized preferentially at a molecular weight range of 1000–3000. Well characterized polypeptide growth inhibitors like interferons, tumor necrosis factor α and β, and the tumor growth factor β family (30–32) would not be involved, taking into account their larger molecular weight and other physical properties (e.g., tumor necrosis factors and interferons do not resist boiling); in addition, no interferon activity was detected in LB serum using a cytopathic assay. However, further characterization and purification of this growth-inhibitory activity will be necessary to evaluate accurately its relation, if any, with these and other previously reported tumor growth-inhibitory factors (33). The origin of this peptide(s) remains speculative; up to now we have not been able to recover activity either in conditioned medium of LB cultures or in serum from mice implanted with a huge mass of irradiated LB tumor.

Whatever its chemical nature and origin, experiments using parabiotic mice support the contention that this growth-inhibitory activity could play an etiological role in the induction of concomitant resistance. In effect, resistance to an LB challenge was seen in a normal mouse joined in parabiosis with an LB-bearing one. This mimicry of concomitant resistance was correlated with the presence of seric growth-inhibitory activity transferred from the LB-bearing partner; furthermore, when both partners were separated, the titer of growth-inhibitory activity decayed rapidly in the serum of the non-LB-bearing mouse in which the previously dormant LB cells began to grow. These experiments suggested (a) that concomitant resistance would be dependent on the presence of a high and persistent level of growth-inhibitory activity and (b) that this level could be attained in mice without any apparent illness, the latter implying that this seric growth-inhibitory activity may eventually be of benefit for the therapeutic control of LB tumor.

However, up to now, we have not been able to transfer antitumor resistance by LB serum inoculation, presumably due to the rapid disappearance of growth-inhibitory activity in normal serum. Taking into account that normal serum did not counteract in vitro the effect of LB serum or that clearance functions did not seem to be affected in LB-bearing mice, the most simple explanation for the high and persistent level of growth-inhibitory activity in LB-bearing mice would be that it is continually produced to compensate for a high loss rate; if so, the problem of transferring antitumor resistance would be merely a technical one, based on determining what dose and schedule of LB serum inoculation could mimic its production in vivo. However, the large production of growth-inhibitory activity required by this explanation and the numerous disappointing attempts to transfer antitumor resistance by inoculating serum from tumor-bearing animals in this and other models (34–37) suggest that a new approach should be considered. It is intriguing that there is a similarly puzzling situation in the regulation of liver growth. In effect, partial or total hepatectomy stimulates mitosis in previously resting hepatocytes wherever they are in the body (38–40) in the same way that excision of a primary tumor induces mitosis in a previously arrested secondary implant (12, 41); furthermore, sera from normal and hepatectomized rats exhibit inhibitory and stimulatory effects, respectively, on in vitro proliferation of hepatocytes (42) and characterization of two seric growth-promoting factors have been reported (43, 44); however, numerous attempts to inhibit liver regeneration by repeated injections of normal serum or to stimulate liver growth by injecting serum from hepatectomized rats have been unsuccessful (39, 45), while transfer of inhibitory or stimulatory signals was possible in parabiotic rats (39). If the analogies were more than casual, the understanding of concomitant resistance induced by nonimmunogenic tumors could eventually help to unveil some of the control mechanisms of malignant and normal cell proliferation.

ACKNOWLEDGMENTS

We are grateful to Drs. S. L. Rabasa, M. A. Isturiz, and E. Poskus for constructive suggestions and to J. J. Portaluppi, A. Morales, C. Lanari, M. Giordano, H. Goldman, E. Massouh, D. López, and C. B. Dejean for expert technical assistance.

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