Correlation and modulation of Ehrlich ascites tumor growth with tumor-plasma IgA

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Plasma IgA level of Ehrlich ascites tumor bearing mice showed correlation with progress of tumor growth. In PAGE analysis total plasma IgA separated into 3 major bands corresponding to mol. wt. ≥669,000 daltons, = 443,000 daltons and between 443,000 and 150,000 daltons. All the three bands increased gradually with progress of tumor growth upto day 14 and then declined on day 16. Total plasma IgA isolated by anti-IgA affinity chromatography when adoptively transferred to mice inhibited tumor growth. Affinity-purified plasma IgA separated into three major peak fractions after Sephadex G-200 column chromatography which corresponded with the bands of IgA on PAGE analysis. Three Sephadex G-200 IgA fractions when adoptively transferred to tumor-bearing mice showed effect different from total IgA. High mol. wt. IgA fraction (≥669,000 daltons) inhibited tumor growth whereas medium mol. wt. fraction (=443,000 daltons) enhanced tumor growth. The low mol. wt. IgA fraction (<443,000 and >150,000 daltons) had no significant effect on tumor growth. The high mol. wt. IgA fraction enhanced tumor killing ability of peripheral blood lymphocytes (PBL) and peritoneal macrophages of tumor bearer in vitro. Medium mol. wt. IgA fraction inhibited tumor-killing ability of PBL in vitro but had no significant effect on peritoneal macrophages. The low mol. wt. IgA fraction showed a mild enhancing effect on tumor-killing ability of PBL but had no significant effect on peritoneal macrophages. The results established importance of IgA in tumor growth regulation and its therapeutic potentiality. The results indicated that tumor growth modulation by tumor plasma IgA is also mediated by its effect on cellular anti-tumor immune factors of the host.

When a tumor develops in a host it is recognized as foreign by the immune machinery of the host due to presence of tumor-associated and/or tumor-specific antigens on its surface. The recognition is expressed by the generation of both humoral, i.e., anti-tumor antibodies and cellular components like cytotoxic killer T-lymphocytes, macrophages against the tumor. The humoral immune system of a host, specially IgG and IgM type of antibody against tumor-associated and/or tumor-specific antigens have been established to play a vital role in tumor protective mechanism. IgG as well as IgM type of antibody have been reported in many investigations to give protection to a host against tumor growth⁴-⁸, whereas in some other studies these immunoglobulins, specially IgG type of antibody have been shown to enhance tumor growth⁴-⁸. However, there are very few reports regarding role of IgA in cancer. In fact, in case of most antigens in animals as well as humans the recognition as foreign is first expressed by the generation of IgM type of antibody by developing lymphoid cells of the plasma cell line (B-cells). Then depending on the nature of antigens the antibody production switchovers to IgG class. The cells committed to synthesis of IgA type antibody also arise from the cells that formerly made IgM type of antibody by genetic switchover mechanism following the sequence IgM → IgG → IgA. Antibodies against EAC cells are expected to follow similar sequence.

IgA type of antibody has been suggested to inhibit growth of tumor cells under in vitro culture conditions⁹-¹¹. Serum IgA levels have been shown to increase in human patients with cancer of oral cavity, cervix, breast and nasopharynx¹²-¹⁹. Briesel et al.⁴ have shown that concentration of secretory IgA in circulation of patients with breast cancer increased significantly. In fact, IgA type of antibody predominates in secretory mucosal and glandular sites, but from these sites come to the circulation through lymphatics. There is only one report

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showing tumor-growth regulatory role of IgA in vivo; Voison et al.\textsuperscript{29} reported that growth of sarcoma in Ic mouse could be enhanced by treatment with antigen-antibody complex of IgA. Although all these studies indicated that besides IgG and IgM, IgA type of antibody has some definite role in malignancy, but this role of IgA has not been studied so far in detail. Therefore in the present investigation with Ehrlich ascites tumor model, role of tumor-plasma IgA in tumor growth regulation and therapeutic implication, if any, of this immunoglobulin class has been evaluated.

**Materials and Methods**

**Tumor**—Ehrlich ascites tumor maintained by serial transplantation in adult male Swiss mice by intraperitoneal inoculation of $1 \times 10^6$ viable tumor cells/mouse, was used.

**Plasma**—The plasma were separated by centrifugation from heparinized blood of normal as well as Ehrlich ascites tumor bearing mice on day 2, 4, 6, 8, 10, 12, 14 and 16 after tumor cell inoculation, ip, on day 0.

**Preparation of anti-IgA affinity Column**—Mouse anti-IgA antibody (Sigma Chem. Co., St. Louis) tested to be specific for IgA by immunogel diffusion in agar plate against standard mouse IgA, IgG and IgM was used for the preparation of anti-IgA affinity column.

Freeze-dried powder of CNBr-activated Sepharose 4B (Sigma Chem. Co., St. Louis; 1.5 g) was swollen for 15 min in 5 ml of 1 M HCl and washed on a sintered glass filter (porosity G3) with 300 ml of the same HCl solution. The gel was then washed with 10 ml of 0.1 M NaHCO$_3$ coupling buffer, pH 8.3, containing 0.5 M NaCl, suspended in 7.5 ml of the same buffer and immediately transferred to a stopped glass tube containing 14 mg anti-IgA antibody in 2.5 ml of NaHCO$_3$ coupling buffer and mixed in an end-over-end fashion for 2 hr at room temperature. After 2 hr the gel was poured into the sintered glass filter, washed with 10 ml of 0.1 M HCl, HCO$_3$ buffer, pH 8, a blocking buffer, suspended in the same buffer and kept for 2 hr at room temperature. Thus the remaining active groups in the gel were blocked. To remove the excess unabsorbed protein, if any, the gel was washed alternatively with high and low pH buffer solutions four times. Sodium acetate buffer, 0.1 M, pH 4 and NaHCO$_3$ coupling buffer pH 8.3, each containing 0.5 M NaCl were used for this purpose. The unreacted blocking agent was washed away with NaHCO$_3$ coupling buffer. Anti-IgA coupled gel was poured into a small column, equilibrated with PBS, pH 7.2 and used for affinity separation of IgA from plasma.

**Separation of plasma IgA by anti-IgA affinity column**—Immunoglobulin A (IgA) from 1 ml plasma of normal mice and from 1 ml plasma, each of Ehrlich ascites tumor bearing mice on day 2, 4, 6, 8, 10, 12, 14 and 16 were separated by anti-IgA affinity column. Each 1 ml plasma diluted to 3 ml with PBS, pH 7.2, was added stepwise (1 ml at a time) to the column, allowed to be absorbed for 15 min each time and then the column was washed thrice with 10 ml PBS, pH 7.2 to remove unabsorbed proteins. The bound IgA from affinity column was eluted in a 3 ml fraction with glycine-HCl buffer, pH 2.5 to a total volume of 24 ml. The fractions were pooled and immediately dialysed against 100 ml PBS, pH 7.2 for 24 hr with 2 changes of the dialysing medium. The total volume of the IgA solution was reduced to 0.8 ml and again dialysed against 100 ml PBS, pH 7.2 for 24 hr with 4 changes of the dialysing medium. Then the volume of the IgA solution was adjusted to 1 ml with PBS, pH 7.2, kept below $-2.0^\circ C$ until further analysis.

**Polyacrylamide gel electrophoretic (PAGE) analysis of IgA**—Affinity purified IgA of normal plasma as well as of tumor-plasma on different days of tumor growth were analysed by 7.5% PAGE using 0.2 M tris-glycine, pH 8.5 as electrophoresis buffer at a constant current of 30 mA. The gel was silver stained following the method of Bloom et al.\textsuperscript{31}. Standard proteins of known mol. wt. (thryoglobulin: mol. wt. 669,000 daltons, apoferritin: mol. wt. 443,000 daltons, alcohol dehydrogenase: mol. wt. 150,000 daltons) were run parallel in the gel to ascertain the mol. wt. of the IgA bands.

**Quantitation of IgA bands separated on PAGE using a gel scanner**—Silver-stained IgA bands on PAGE were scanned at 410 nm using a Shimadzu CS-9000 Scanner. The IgA bands separated according to their different mol. wt. were scanned and recorded as different peaks. The peaks as well as the peak areas were printed out by the printer. These peak areas were proportional to the amount of IgA present in the band which indirectly represented the concentration of that IgA in the plasma.

**Adoptive transfer of IgA in mice with tumor cell or after tumor cell inoculation**—Affinity purified tumor plasma IgA in PBS, pH 7.2, was divided into two parts. One part was mixed with Ehrlich tumor cells in PBS, pH 7.2 and injected, ip, to each
mouse of a group of 4 mice on day 0, so that each mouse received 250 μg of IgA and 1×10⁵ tumor cells in 0.5 ml PBS. A second group of 4 mice received 1×10⁵ tumor cells/mouse, ip, in 0.5 ml PBS on day 0 and 250 μg IgA in 1 ml PBS/mouse, ip, on day 1, 2 and 3 from the other part of IgA solution. The control group received only 1×10⁻⁴ tumor cells/mouse, ip, on day 0. Another group of mice received IgA purified from normal mouse plasma in a similar manner as in group 1. This was IgA control for group 1. On day 4 after tumor inoculation tumor cells were washed out from the peritoneal cavity of each mouse with 5 ml PBS, pH 7.2. The tumor cell count/ml was determined by trypsin blue dye exclusion technique and the total count was obtained by multiplying the count/ml with volume.

Separation of affinity purified tumor plasma IgA by Sephadex G-200 column chromatography—Affinity purified tumor-plasma IgA separated into three peak fractions (1, 2 and 3) when passed through a Sephadex G-200 column, equilibrated and eluted with PBS, pH 7.2 into 5 ml fractions. Three peak fractions 1, 2 and 3 corresponded with the mol. wt. standards, thyroglobulin (669,000 daltons), apoferritin (443,000 daltons) and alcohol dehydrogenase (150,000 daltons) respectively run in the same column. Three fractions gave precipitin bands against anti-IgA antibody in immunogel diffusion study.

Adoptive transfer of Sephadex G-200 IgA-fractions in mice after tumor cell inoculation—Three groups of mice received b×10⁵ tumor cells/mouse, ip, in 0.5 ml PBS on day 0. Then these three groups received Sephadex G-200 IgA-fractions 1, 2 and 3 respectively in various doses, ip, on day 1, 2 and 3. The control group received only 1×10⁵ tumor cells/mouse, ip, on day 0. Tumor cell counts of each mouse of each group were taken on day 4 using trypsin blue dye exclusion technique.

₅¹Cr-release assay to study the in vitro tumor-killing by peripheral blood lymphocytes (PBL) and peritoneal macrophages—Peripheral blood lymphocytes (PBL) were separated from heparinized blood (diluted 1:1 with RPMI-1640 medium) of day-6 tumor bearing mice by centrifugation over Ficoll Hypaque layer at 400 g for 30-40 min at 18°-20° C. The buffy coat of lymphocyte at the interface was removed with a pasteur pipette, washed thrice with 5 volumes of medium, counted in a haemocytometer and diluted to the desired concentration with medium (+10% FBS).

Peritoneal macrophages were collected from day-6 tumor-bearing mice by washing with heparinized PBS, pH 7.2 and centrifuged. The cells were suspended in 5 ml RPMI-1640 (+10% FBS) medium and immediately transferred to a petri-dish and incubated for 1 hr at 37°C in a 5% CO₂-incubator. The supernatant poured off, added 5 ml PBS containing 10⁻⁴ M EDTA and incubated at room temperature for 10 min. The cells were dislodged by gentle scraping with EDTA-PBS, pH 7.2 and finally suspended in RPMI-1640 (+10% FBS) medium.

Ehrlich tumor cells collected from ascites fluid of Ehrlich ascites tumor-bearing mice were washed thrice with PBS, pH 7.2, then twice with RPMI-1640 medium (+10% FBS) and adjusted to a concentration of 2×10⁵/ml. From this suspension 1 ml was labelled with 300 μci of ⁵¹Cr for 2 hr in a CO₂-incubator with occasional mixing, centrifuged at 800 g for 10 min and washed 5 times with RPMI-1640 medium to remove unlabelled ⁵¹Cr. Finally the cells were counted and kept at 4°C.

To 10 μl of IgA solution (1.5 nmole of each fraction) in each well of a microtitre plate was added 5×10⁴ ⁵¹Cr-labeled tumor cells in 50 μl RPMI-1640 medium (+10% FBS) and incubated for 30 min at 30°C. Then 5×10⁵ lymphocytes or macrophages in 50 μl RPMI-1640 complete medium was added. Additional medium was added to make the volume up to 150 μl, centrifuged at room temperature for 3 min at 80 g to facilitate cell contact; then incubated for 4 hr at 37°C in a CO₂-incubator. Finally added 50 μl ice cold PBS and centrifuged at 400 g for 10 min at 4°C. From each well 100 μl supernatant was carefully removed, transferred to Bio-vial and counted in a gamma counter. Cent per cent release was determined by adding 50 μl of 1% triton x-100 solution to the cell suspension (5×10⁶) in medium. The percent killing was determined using the following formula:

\[
\text{Per cent killing} = \left( \frac{\text{₅¹Cr - label released in the experimental}}{100\% \text{ ₅¹Cr - released}} \right) \times 100
\]

- Count of the experimental
- Count due to spontaneous release \times 100
- Count due to Triton X - 100 treatment
- Count due to spontaneous release

Statistical significance was determined by Student's t test in all the experiments.
Results

Changes in plasma IgA with progress of Ehrlich ascites tumor growth—Affinity purified plasma IgA on different days of tumor growth, e.g., day 2, 4, 6, 8, 10, 12, 14 and 16, as analysed by PAGE has been presented in Fig. 1. PAGE analysis revealed that plasma IgA of Ehrlich tumor-bearing mice separated into 3 major bands (I, II and III). When these bands were compared with standard mol. wt. markers like thyroglobulin (mol wt. 669,000 daltons), apoferritin (mol. wt. 443,000 daltons) and alcohol dehydrogenase (mol. wt. 150,000 daltons) run parallel to these IgA samples, indicated that band I had mol. wt. ≥ 669,000 daltons, band II mol wt. around 443,000 daltons and band III mol. wt. between 443,000 and 150,000 daltons. Moreover, staining intensity of the IgA bands suggested quantitative changes in all the three types of IgA bands with progress of tumor growth.

The concentration of plasma IgA bands on polyacrylamide gel were determined by scanning with a gel scanner. The concentrations of different mol. wt. IgA on different days of tumor growth as expressed by different peak profiles have been presented in Fig. 2. The quantitative data as calculated from the peak areas for different mol. wt. IgA on different days of tumor growth have been presented in Fig. 3. Both Figs 2 and 3 confirmed the visual observation on PAGE. Total plasma IgA of day 2 tumor bearing mice was 1.13 times higher than that of normal plasma and the level gradually increased with progress of tumor

Fig. 1—Affinity purified plasma IgA on different days of tumor growth, e.g., day 2, 4, 6, 8, 10, 12, 14 and 16, as analysed by polyacrylamide gel (7.5%) electrophoresis (PAGE) using 0.2 M Tris-glycine, pH 8.5 as electrophoresis buffer at a constant current of 30 mA and silver stained. Standard proteins of known mol. wt., e.g., thyroglobulin (mol.wt. 669,000 daltons), apoferritin (mol. wt. 443,000 daltons) and alcohol dehydrogenase (mol. wt. 150,000 daltons) were run parallel to ascertain the mol. wt. of the IgA bands separated on PAGE (S); N: IgA from normal mouse plasma.

Fig. 2—Concentration profiles of plasma-IgA of different mol. wt., separated on PAGE for different days of tumor growth, as scanned by a gel scanner at 410 nm.
growth. On day 14 of tumor growth the level became almost 3.5 times the normal value and subsequently decreased on day 16 to a level of 2.44 times the normal value. The levels of three major IgA bands in the tumor plasma also increased gradually with progress of tumor growth. The levels were maximum on day 14 of tumor growth, about 2.6 times the normal value for IgA of mol. wt. ≥ 669,000 daltons, 4 times for IgA of mol. wt. = 443,000 daltons and 4.2 times for IgA of mol. wt. between 443,000 and 150,000 daltons. The levels of high, medium and low mol. wt. IgA came down to a value of 1.6, 2.5 and 3.4 times the normal value respectively on day 16 of tumor growth.

Effect of adoptively transferred tumor plasma IgA on Ehrlich ascites tumor growth—When affinity purified plasma IgA of Ehrlich ascites tumor-bearing mice was adoptively transferred with tumor cells (1×10⁷/mouse) to normal mice on day 0, ip, and the tumor cell counts were taken on day 4, the number of tumor cells were found to decrease significantly (55%, P < 0.01) compared to control mice, receiving only tumor cells on day 0 (Table 1). This inhibitory effect was dose dependent and the maximum effect obtained at 250 µg of IgA has been presented in Table 1. In another group of mice receiving tumor cells on day 0, ip, and tumor plasma IgA, ip, on day 1, 2 and 3, tumor cell counts were also observed to decrease significantly (56%, P < 0.01) compared to control (Table 1). This was also a dose dependent effect and the maximum effect was obtained at 250 µg of IgA. The control group receiving IgA from normal mouse plasma showed tumor cells counts almost same as that in control group receiving only tumor cells (Table 1, Expt. 2), i.e., normal mouse plasma IgA had no effect on Ehrlich ascites tumor growth.

Effect of adoptively transferred tumor-plasma IgA of different mol. wt. on Ehrlich ascites tumor growth—Affinity purified total IgA of tumor-plasma was separated by Sephadex G-200 column chromatography into its various mol. wt. fractions as shown in Fig. 4. Three peak fractions (Fracl, Frac. 2 and Frac. 3) were obtained which corresponded with the standard mol. wt. markers thyroglobulin (660,000 daltons), apoferritin (443,000 daltons) and alcohol dehydrogenase (150,000 daltons) respectively, run in the same column. Three fractions were tested for their effect on Ehrlich tumor growth. The fractions showed effect different from total plasma IgA. The high mol wt. fraction (≥ 669,000 daltons) when injected, ip, on day 1, 2, and 3 after tumor cell inoculation on day 0, ip, inhibited tumor growth.

![Fig. 3—Plasma IgA levels on different days of Ehrlich ascites tumor growth. 2T, 4T, ⋯ ⋯ 16T represent total plasma IgA on respective days of tumor growth. 2L, 4L, ⋯ ⋯ 16L: 2M, 4M, ⋯ ⋯ 16M and 2H, 4H, ⋯ ⋯ 16H represent concentration of low, medium and high mol. wt. plasma IgA respectively on respective days of tumor growth. Bar heights are directly proportional to the peak areas of the respective IgA bands on polyacrylamide gel, scanned by a Gel-scanner, which in turn are proportional to the concentration of the respective IgA in plasma. N₁, N₂, N₃ and N₄ represent total, low mol. wt., medium mol. wt. and high mol. wt. IgA of normal mouse plasma. N₁, N₂ and N₃ are 31.5, 29.0 and 39.5 percent respectively of N₁ as determined from their scanned peak area values. Number on each bar means corresponding normal bar value multiplied by that number represent that particular bar value. Bar height of N₁ = 30 mm (arbitrarily chosen). Results of one from two representative experiments have been presented.](image-url)
growth significantly (55%, *P*<0.0005) and the inhibition was dose dependent. The maximum inhibition was obtained at 250 μg IgA/mouse (Table 2). The IgA of medium mol wt. (= 443000 daltons) enhanced tumor growth in a dose dependent manner and the maximum effect was obtained at 250 μg IgA/mouse (39%, *P*< 0.0005). However IgA of low mol. wt. (443,000-150,000 daltons), showed no effect on tumor growth (Table 2).

Survival of the group receiving high mol. wt. IgA was 100% on day 22 when all the mice of control were dead (data not shown).

**Effect of different mol. wt. plasma-IgA of Ehrlich ascites tumor-bearing mice on the tumor killing ability of peripheral blood lymphocytes (PBL) and peritoneal macrophages of the same tumor-bearer in vitro**—The tumor killing ability of PBL and peritoneal macrophages of Ehrlich tumor-bearing mice was studied in vitro by 51Cr-release assay in presence of high mol. wt. IgA-fraction (≥ 669,000 daltons), medium mol. wt. IgA-fraction (=443,000 daltons) and low mol. wt. IgA fraction (443,000-150,000 daltons), obtained from Ehrlich tumor-plasma by Sephadex G-200 column chromatography. The high mol. wt. IgA-fraction added at a dose of 1.5 nmole enhanced tumor-killing ability of PBL by approximately 161%, whereas the same dose of medium mol. wt. IgA-fraction inhibited tumor-killing ability of PBL by 32.5%. The low mol. wt. IgA-fraction enhanced tumor-killing ability of PBL only by 25% (Table 3).

The high mol. wt. IgA-fraction added at a dose of 1.5 nmole enhanced tumor-killing ability of peritoneal macrophages by approximately 130%. The medium mol. wt. IgA-fraction at the same dose inhibited tumor-killing ability of macrophages only by 3% which was not statistically significant. Similarly low mol. wt. IgA-fraction enhanced tumor-killing ability of macrophages only by 5.5% which was also not statistically significant (Table 3). The dose of 1.5 nmole was the optimum dose to show maximum effect in case of both PBL and peritoneal macrophages. Normal plasma-IgA showed no effect on PBL and peritoneal macrophages (data not shown).

**Discussion**

Using Ehrlich ascites animal tumor model in the present investigation it has been established that there is definitely a correlation between plasma IgA level and progression of tumor growth. Plasma IgA level gradually increased up to day 14 of tumor growth, then decreased on day 16 when the animals began to die due to tumor burden. Similar type of correlation between tumor growth and plasma IgA level was observed in studies with 3-methylcholanthrene induced fibrosarcoma bearing C3H/HeJ mice. Also plasma IgG and IgM levels of fibrosarcoma-bearing

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**Table 1**—Effect of adoptively transferred tumor-plasma IgA on Ehrlich ascites tumor growth

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Expt. No.</th>
<th>Count of tumor cells (x10⁵) on day 4 after tumor cell inoculation on day 0</th>
<th>Tumor inhibition (%)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Received 1x10⁵ tumor cell + 250μg IgA from tumor plasma/mouse, ip, on day 0</td>
<td>1</td>
<td>38.5±14.7</td>
<td>50.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>19.6±2.9</td>
<td>58.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Received 1x10⁵ tumor cells/mouse, ip, on day 0 and 250μg IgA from tumor plasma/mouse, ip, on day 1, 2 &amp; 3</td>
<td>1</td>
<td>29.4±11.3</td>
<td>62.5</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>24.3±2.3</td>
<td>49.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>48.0±0.9</td>
<td>56.0</td>
</tr>
<tr>
<td>Received 1x10⁵ tumor cells + 250μg IgA from normal mouse plasma, ip, on day 0</td>
<td>1</td>
<td>ND</td>
<td>ND</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>46.7±1.6</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Received only 1x10⁵ tumor cells/mouse, ip, on day 0 (control)</td>
<td>1</td>
<td>78.4±11.4</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>47.8±9.9</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>99.6±21.5</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND = not done; NS = not significant.
Table 2—Effect of tumor-plasma IgA of different mol. wt. on the growth of Ehrlich ascites tumor

<table>
<thead>
<tr>
<th>Treatment: IgA injected i.p. on day 1, 2 and 3 to groups of mice receiving tumor cells (1x10⁵/mouse, i.p.) on day 0.</th>
<th>Group</th>
<th>Doses Of IgA (µg)</th>
<th>Tumor cell count (x 10⁵) on day 4 after Tumor cell inoculation on day 0.</th>
<th>Significance P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>High mol. wt. IgA-fraction (≥ 669,000 daltons)</td>
<td>1.</td>
<td>250</td>
<td>20.67±3.63 (51.0)</td>
<td>&lt;0.0005 for Expt. 1, 2 &amp; 3</td>
</tr>
<tr>
<td>Medium mol. wt. IgA-fraction (≈ 443,000 daltons)</td>
<td>1.</td>
<td>250</td>
<td>56.80±3.81 (34.6)</td>
<td>&lt;0.0005 for Expt. 1 &amp; 3</td>
</tr>
<tr>
<td>Low mol. wt. IgA-fraction (443,000 to 150,000 daltons)</td>
<td>1.</td>
<td>250</td>
<td>41.40±5.31 (11.9)</td>
<td>&lt;0.0005 for Expt. 2</td>
</tr>
<tr>
<td>No IgA injected. Received only 1x10⁵ tumor cells/mouse, i.p. on day 0 (Control)</td>
<td>C</td>
<td>--</td>
<td>42.20±2.39</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

ND = not done; NS = not significant.

Table 3—Effect of different mol. wt. plasma IgA-fractions of Ehrlich tumor-bearing mice on the tumor killing ability of peripheral blood lymphocytes (PBL) and peritoneal macrophages of the same tumor-bearer studied in vitro by ⁵¹Cr-release assay

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Sephadex G-200 Percent tumor killing</th>
<th>Percent Enhancement (En) or Inhibition (In) in tumor killing by IgA fractions</th>
<th>Significance P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of tumor cells/No. of lymphocytes</td>
<td>1.</td>
<td>5x10⁷/5x10⁵ (1:100)</td>
<td>Nil</td>
</tr>
<tr>
<td>2.</td>
<td>5x10⁷/5x10⁵ (1:100)</td>
<td>Frac 1 (tetrameric and/or higher polymeric-lgA)</td>
<td>44.80±7.61</td>
</tr>
<tr>
<td>3.</td>
<td>5x10⁷/5x10⁵ (1:100)</td>
<td>Frac 2 (trimeric-lgA)</td>
<td>14.45±3.20</td>
</tr>
<tr>
<td>4.</td>
<td>5x10⁷/5x10⁵ (1:100)</td>
<td>Frac 3 (di- and/or monomeric-lgA)</td>
<td>24.85±1.17</td>
</tr>
<tr>
<td>No. of tumor cells/No. of macrophages</td>
<td>1.</td>
<td>5x10⁷/5x10⁵ (1:100)</td>
<td>Nil</td>
</tr>
<tr>
<td>2.</td>
<td>5x10⁷/5x10⁵ (1:100)</td>
<td>Frac 1 (tetrameric and/or higher polymeric-lgA)</td>
<td>40.94±3.93</td>
</tr>
<tr>
<td>3.</td>
<td>5x10⁷/5x10⁵ (1:100)</td>
<td>Frac 2 (trimeric-lgA)</td>
<td>23.12±4.93</td>
</tr>
<tr>
<td>4.</td>
<td>5x10⁷/5x10⁵ (1:100)</td>
<td>Frac 3 (di- and/or monomeric-lgA)</td>
<td>24.77±0.60</td>
</tr>
</tbody>
</table>
mice increased with progress of tumor growth compared to normal control mice but did not show any linear correlation with tumor growth\(^1\). Therefore in the present investigation with Ehrlich ascites tumor model we became interested about plasma IgA level but not plasma IgG or IgM levels. Moreover, it has been shown in the present investigation that IgA, obtained from the plasma of Ehrlich ascites tumor-bearing mice when adoptively transferred to normal mice with tumor cells or after tumor cell inoculation could inhibit subsequent tumor growth significantly (55-56%). So far tumor inhibitory antibodies have been shown to be of IgG or IgM type\(^1,4,23\). However, for the first time in the present study, it has been shown that IgA type of antibodies may have similar effect. In the present study normal mouse plasma IgA did not show any tumor growth inhibitory effect, suggesting that tumor-growth inhibitory effect is possibly due to the presence of tumor-specific IgA-type antibody in IgA preparation obtained from tumor-plasma. This results as well as results reported with human cancer patients\(^9,11,15,19\) suggest that IgA level in the circulation may be a good marker for diagnosis as well as prognosis of tumor.

The more interesting observation is that while total plasma IgA transferred adoptively inhibited tumor growth, IgA-fractions separated from tumor-plasma on the basis of mol. wt. acted differently. High mol. wt. IgA-fraction (≥669,000 daltons) inhibited tumor growth whereas medium mol. wt. IgA-fraction (=443,000 daltons) enhanced tumor growth. The low mol. wt. IgA-fraction (between 443,000 and 150,000 daltons) had no effect on tumor growth. Taking into consideration the mol. wt. of monomeric IgA as 160,000 daltons, the high mol. wt. fraction is suggested to be tetramer and/or higher polymers of IgA, the medium mol. wt. fraction to be trimeric IgA and the low mol. wt. fraction to be dimeric and/or monomeric IgA.

At present it is not sure why tetra and/or higher polymers of IgA and trimeric IgA are exerting totally opposite effect on tumor growth and what are their mechanism of action. However, in one mechanism it was suggested that tumor escapes immune destruction by the host by suppressing anti-tumor immune responses of the host by blocking antibodies\(^5,8,24,25\). So, the medium mol. wt. IgA-fraction, i.e. trimeric IgA, showing tumor enhancement in vivo may be blocking antibodies. This view is supported by the observation that trimeric IgA-fraction of tumor-plasma inhibited tumor-killing ability of PBL and peritoneal macrophages of tumor-bearer in vitro. In another mechanism it was suggested that tumor escapes immune destruction by the host by suppressing the immune reaction of the host against the tumor. This occurs through blocking factors which are suggested to be tumor-specific and/or tumor-associated antigens. Tumors constantly shed such antigens into the circulation which form complexes with the tumor-specific antibodies and thus precipitate the anti-tumor antibodies in the circulation before they can reach the tumor cells and are eliminated from the circulation in due course\(^5\). Similar mechanism may be operative in tumor specific IgA molecules.
present in the circulation of EAC-bearing mice. They
are precipitated in the circulation before they can
reach the intraperitoneal cavity. On the other hand,
the IgA molecules adoptively transferred with EAC
cells are directly coming in contact with the tumor
cells and in combination with macrophages and/or
lymphocytes are killing the tumor cells before they
are precipitated by the antigens shed by the tumor
cells. The high mol. wt. IgA-fraction, i.e., tetrameric
and/or, higher polymeric IgA-fraction showing tumor
growth inhibition in vivo, may be anti-tumor antibody.
Moreover, this tetrameric and/or, higher polymeric
IgA-fraction enhanced tumor-killing ability of PBL
and peritoneal macrophages of tumor-bearer in vitro,
suggesting that anti-tumor effect of this high mol. wt.
IgA-fraction is also mediated by enhancing the tumor-
killing ability of cellular anti-tumor factors of the
host. Role of low mol. wt. IgA-fraction, i.e., di-
and/or, monomeric forms of IgA in vivo is not clear,
although its level in the circulation increased with
progress of tumor growth and this fraction of IgA
showed mild enhancing effect on the tumor-killing
ability of PBL in vitro but had no significant effect on
peritoneal macrophages. Possibly this low mol. wt.
IgA-fraction has some additive effect to the tumor-
killing ability of PBL, besides high mol. wt. IgA-
fraction.

In conclusion it is worth mentioning that quite
opposite effect of IgA of different mol. wt. on tumor
growth indicates that proper manipulation of these
biomolecules may have potential therapeutic
application. However, before considering therapeutic
application, above information should be established
with etiologically defined solid animal tumor models.
Besides its therapeutic application IgA may also have
its diagnostic as well as prognostic application. In
fact, Iglehart et al. established correlation between
secretory immune system, namely IgA and bronchogenic
carcinoma and suggested that measurement of secretory IgA may be useful in the early diagnosis of malignant bronchogenic diseases and they also suggested clinical application of IgA. Similarly, Khalifa et al. suggested use of IgA as
tumor-markers in bladder cancer of humans. Zong
et al. suggested use of IgA antibody against viral
capsid antigen of Epstein-Barr virus (EBV) for
screening of early stage of nasopharyngeal carcinomas, as they observed the presence of this
antibody in the blood of many subjects during
screening of a population in southern China. Petrelli
et al. demonstrated the clinical utility of serum IgA-

as a potential complementary tumor markers to
CEA in the monitoring of the post-operative course of
patients with advanced colorectal adenocarcinoma.

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