Effect of Tumors on the Concentration of Leucogenenol in the Serum of Mice

F. A. H. Rice, Christian Oresajo, J. R. Heath, E. J. Breyere, and J. D. McCurdy

ABSTRACT

It was found that inoculation of several strains of mice with several types of tumor cells resulted, within 24 hr, in a significant decrease in the serum leucogenenol levels of the mice. Serum leucogenenol levels of the mice inoculated with tumors that are rejected become normal or temporarily above normal at approximately the time the tumor is observed to be rejected. Contrariwise, serum leucogenenol levels of mice inoculated with tumors that are not rejected remain at significantly lower than normal levels during the life of the mice. Unlike tumors, skin allografts increase serum leucogenenol levels.

When tumors are rejected because of the previous immunization of the mice, serum leucogenenol levels become normal at approximately the time the tumor is observed to be rejected.

Excision of the tumor after 1 week of growth, with the consequent recovery of the mice, is accompanied by a recovery of normal serum leucogenenol levels.

Also, it was found that injection of mice with a cell-free 0.9% NaCl solution extract of a tumor results in a temporary decrease in serum leucogenenol levels comparable to that observed with the inoculation of a viable tumor which lasts from 24 to 96 hr.

It is suggested that the suppression of serum leucogenenol levels is one of the factors responsible for the immunosuppression associated with a growing tumor.

INTRODUCTION

Leucogenenol, 2-(1,2-dihydroxy-3-methyl-5-oxocyclohexyl)-3,11-dihydroxy-11-(hydroxymethyl)-9-methyl-1-oxa-5-azaaspiro[5.5]undeca-2,4-dien-7-one (9), is a thymothyroid hormone, which is isolated from liver (21), thymus, thyroid, adrenals, and gonads (22) in quantities of approximately 5 µg/g of dry tissue. It is present in lesser quantities in lymphoid tissue, bone marrow, and blood serum. In blood serum, it is associated with a specific carrier protein (16). The concentration of leucogenenol in the serum of animals varies with the species. Adult rabbits (11) have 52 ± 9 µg, adult rats (11) 35 ± 9 µg, and adult male humans 8 ± 4 µg of leucogenenol per liter in their serum (18). Human females have 20 ± 4 µg of leucogenenol per liter in their serum during menstruation which drops to 10 ± 4 µg/liter 14 days later.3

Temporary changes in serum leucogenenol levels are associated with a number of abnormal physiological conditions. Twenty-four hr following a skin or muscle incision or the loss of blood, rats and rabbits show elevated levels of serum leucogenenol which return to normal in approximately 4 days (16).

Likewise, patients with rheumatoid arthritis have 4- to 5-fold elevated levels of serum leucogenenol which become essentially normal during periods of remission.3 Dogs, following lethal y-irradiation from 60Co, have depressed serum leucogenenol levels. Indeed, serum leucogenenol is not detectable approximately 4 days before the dog dies (11).

We wish to report that serum leucogenenol levels are also affected by the transplantation of tumors into mice.

MATERIALS AND METHODS

Animals, Tumors and Leucogenenol Assay. BALB/c mice, C57BL/B6 mice, BALB/c, DBA/2, and C57 mice, were obtained from colonies maintained at The American University (E. J. Breyere), DBA-49, a sarcoma of BALB/c origin, which grows progressively in normal BALB/c and C57 mice, SS(70429), a plasma cell tumor of C3H origin, and C77, a teratoma of BALB/c origin, were obtained from tumors also maintained at The American University (E. J. B.). BALB/c × DBA/2 F(2)(hereafter called CD2F) mice and L1210 tumor cells were obtained respectively through the courtesy of C. Gallo and Dr. Richard Wood of the National Cancer Institute, Bethesda, Md. The L1210 tumor cells were maintained in the peritoneal cavity of CD2F, mice.

Unless otherwise stated, mice were inoculated s.c. in the right flank with 0.025 ml of a suspension of tumor cells containing approximately 3 × 10^6 viable cells (trypan blue exclusion method) prepared by forcing freshly excised tumor tissue through a tissue press and then diluting it with 0.9% NaCl solution (approximately equal volume) to yield the desired concentration of cells.

Suspensions of L1210 cells were prepared by aspirating approximately 2 ml of fluid from the peritoneal cavity of tumor-bearing CD2F mice, separating the cells by centrifugation (3000 rpm in an International Clinical Centrifuge) and washing them twice (2 ml) with 0.9% NaCl solution by centrifugation. The cells were then resuspended in 0.9% NaCl solution to yield an approximate concentration of 10^6 viable cells (trypan blue exclusion method) injection of 0.025 ml.

Blood was obtained from the mice by cardiac puncture under ether anesthesia.

The concentration of leucogenenol in the serum was determined by the method reported previously (18). Briefly, the leucogenenol is quantitatively isolated from a known volume of serum (approximately 1 ml) and dissolved in 4 ml of Fisher's medium for leukemic cells of mice (Grand Island Biological Co., Grand Island, N.Y.) buffered (pH 7.2) with 4-(2-hydroxyethyl)-1-piperazineethane-sulfonic acid. Serial 10-fold dilutions are then made, and the change in the respiratory quotient of L5178 cells due to the addition of each of at least 4 dilutions was determined with a Gilson respirometer. The quantity of leucogenenol added to the medium affects the respiratory quotient of L5178 cells in a manner represented by a normal distribution curve (18) with a maximum increase in respiratory quotient (approximately 2-fold) when 1.92 × 10^-4 µg of leucogenenol is added to the medium. Since the dilution that causes a maximum change in respiratory quotient is readily calculated from the change in respiratory quotient at each of 3 dilutions (18) and the quantity of leucogenenol that causes this maximum change is known, the concentration of leucogenenol in the serum is easily calculated (18).
Since it was demonstrated that the final chromatographic step in the isolation of leucogenenol from normal serum could be omitted without affecting the results of the assay (18), this last step is usually omitted. However, to assure that no interfering compounds are present when this step is omitted in studies such as reported here, additional assays are performed in which the leucogenenol in a sample of serum is chromatographically isolated, and results are compared with assays on the same serum sample in which the final chromatographic step is omitted. Thus far, no significant differences have been found.

Standard deviations are calculated from 4 determinations.

Concentration of Leucogenenol in the Serum following Inoculation of Tumor Cells. Forty approximately 6-week-old female BALB/c mice were randomly separated into 5 cages. Thirty-four of the mice were then inoculated with DBA-49 tumor cells. Twenty-four hr after inoculation, 6 of the tumor-injected mice and 6 of the untreated mice were exsanguinated. The blood from the tumor-injected mice was combined, the serum was separated, and 1.0-ml aliquots were used to determine its concentration of leucogenenol. Likewise, the blood from the untreated mice was combined, the serum was separated, and its concentration of leucogenenol was determined. At weekly intervals for 5 weeks after the inoculation of the tumor cells, 6 of the tumor-bearing mice were exsanguinated, their blood was combined, the serum was separated, and its concentration of leucogenenol was determined. The experiment was repeated.

In an identical manner, the effect of the injection of tumor cells on serum leucogenenol levels was determined for the following: (a) approximately 6-week-old female BALB/c mice inoculated with SS tumor cells and approximately 6-week-old female BALB/c mice inoculated with C77 tumor cells; (b) approximately 7-week-old male CD2F1 mice inoculated with DBA-49 tumor cells; (c) approximately 7-week-old male CD2F1 mice inoculated with SS tumor cells; (d) 2- to 4-month-old male and female (equal numbers) C57 mice inoculated with SS tumor cells; and (e) approximately 6-week-old female C3H mice inoculated with SS tumor cells.

Thirty-two female and 13 male DBA mice, approximately 3 months old, were randomly separated into groups of 5 mice each. Each mouse in one group of mice was then exsanguinated, their blood was pooled, the serum was separated, and 1.0-ml aliquots were used to determine the concentration of leucogenenol in the serum. Twenty of the mice were then inoculated with SS tumor cells. Twenty-four hr and at weekly intervals after inoculation, 5 mice that had been inoculated with DBA-49 tumor cells were exsanguinated, and the concentration of leucogenenol in their serum was determined as above. Likewise, at the same time intervals, the concentration of leucogenenol in the serum of 5 mice from the group that had been inoculated with SS tumor cells was determined.

Forty male CD2F1 mice, were inoculated s.c. in the right flank with L1210 tumor cells. At 0 hr, 24 hr, 4 days, and 11 days, groups of 6 mice were exsanguinated, and the concentration of leucogenenol in their serum was determined as described above. Due to the death of the mice, samples of blood could not be obtained after 11 days.

Immunization. Each of 42 female 6-week-old BALB/c mice was given s.c. injections in 2 sites, approximately 1 cm apart over the rib cage on the left side, of 0.025 ml of pooled blood freshly obtained from 6 female DBA mice approximately 6 months of age. The inoculation was repeated 9 days later. Eleven days after the second inoculation, 6 of the mice were exsanguinated, their blood was pooled, the serum was separated, and the concentration of leucogenenol in it was determined. The remaining 36 mice were inoculated with DBA-49 tumor cells as described previously, and the concentration of leucogenenol in the serum of groups of 6 mice was determined at 24 hr and at weekly intervals thereafter for 5 weeks as described above.

Tumor Excision. Each of 24 female BALB/c mice (6 weeks old) was inoculated with DBA-49 tumor cells. One week later, the tumors were removed from 12 of the mice by lifting the tumor in a skin pouch, ligating and then excising the pouch, and thus excising the tumor. The remaining 12 mice (control group) had a similar pouch formed of skin and fascia on the left side, opposite the tumor which was ligated and excised. Approximately 0.25 ml of blood was removed from each mouse by cardiac puncture at the time the tumors were removed and at weekly intervals thereafter for 5 weeks. The blood from the control group was pooled, and the serum was separated and assayed for its leucogenenol concentration. Likewise, the blood from the group whose tumors had been excised was pooled, and the serum was separated and assayed for its concentration of leucogenenol.

Skin Allografts. Each of 18 female BALB/c mice were given i.p. injections of 0.1 ml of valum (diazepam; Hoffmann-LaRoche Inc., Nutley, N. J.) containing 5 mg/ml. Anesthesia was completed 15 to 20 min later with the aid of ether and a circular area of skin, approximately 2 cm in diameter, removed with microdissecting scissors. The area from which the skin was removed then was covered with a corresponding patch of skin freshly removed from a C57 mouse anesthetized previously with valum and ether. The replaced skin was covered with an approximately 2.5-cm sterile gauze square moistened with Vaseline petroleum jelly on top of which was placed a second sterile 2.5-cm gauze square. The skin and gauze squares were held in place with a 2.5-cm-wide Band-Aid which circled the animal. The Band-Aid strip then was covered with 2 to 3 wrappings of gauze which was held in place by rayon adhesive tape. At intervals of 1, 4, and 9 days after skin grafting, each of 6 animals was exsanguinated by cardiac puncture under ether anesthesia, and the blood of the 6 animals was pooled. The serum was separated, and its concentration of leucogenenol was determined.

Eighteen female BALB/c mice were treated in an identical manner without, however, transferring the skin from the C57 mouse to the BALB/c recipient.

Tumor Extract. DBA-49 tumors at their fourth week of growth were excised from 15 BALB/c female mice and forced through a tissue press, and 12 ml of the resulting suspension were diluted with 6 ml of 0.9% NaCl solutions. The cells were removed by centrifugation at 3000 rpm (International Clinical Centrifuge), and the clear supernatant was filtered through a Millipore filter (0.2 μm). An amount of 0.05 ml of the fluid was then injected s.c. in the right flank of 24 female BALB/c mice approximately 6 weeks old. At intervals of 24 hr, 1 week, 2 weeks, and 3 weeks thereafter, 6 of the mice were exsanguinated, their blood was pooled, the serum was separated, and its concentration of leucogenenol was determined. The experiment was repeated with the modification that, in place of DBA-49 tumors, the spleens of 15 DBA mice were extracted with 0.9% NaCl solution in the same manner as the tumors and used for injection.

An approximate size of the tumor was obtained by measuring the short and long axes of the elliptically growing tumor with calipers and multiplying these values to give an approximate area (sq cm). Tumors whose area could not be measured in this manner were recorded as: B, no palpable tumor; C, palpable but too small to measure (less than 0.5 cm on one axis); D, tumor too diffuse and disseminated to measure. These tumors grew progressively to finally cover an area estimated to be at least 4 sq cm.

Student’s t test was used to evaluate results statistically.

RESULTS AND DISCUSSION

Table 1 shows that inoculation of mice with any of the tumor cells investigated results in a significant decrease in serum leucogenenol levels 24 hr later. No statistically significant correlation is observed between the degree of depression of serum leucogenenol levels and the size of the growing tumor. For example, in the case of a relatively slow-growing tumor typified by DBA-49 in BALB/c mice, although serum leucogenenol levels further decline the week following the 24-hr period, at a time the tumor is measurable, they do not show further significant changes during the life of the animal. In the case of a more rapidly growing tumor, typified by DBA-49 in DBA mice,

Concentration of Leucogenenol in Mouse Serum
Table 1
Concentration of leucogenenol in the serum of several strains of mice following their inoculation with several types of tumors

<table>
<thead>
<tr>
<th>Strain of mouse</th>
<th>Tumor</th>
<th>Untreated controls</th>
<th>24 hr</th>
<th>1 wk</th>
<th>2 wk</th>
<th>3 wk</th>
<th>4 wk</th>
<th>5 wk</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/c&lt;sup&gt;a&lt;/sup&gt;</td>
<td>DBA-49</td>
<td>50 ± 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28 ± 1&lt;sup&gt;c&lt;/sup&gt; (C)</td>
<td>10 ± 4&lt;sup&gt;c&lt;/sup&gt; (0.70 ± 0.2)</td>
<td>9 ± 1&lt;sup&gt;c&lt;/sup&gt; (1.3 ± 0.3)</td>
<td>10 ± 2&lt;sup&gt;c&lt;/sup&gt; (2.1 ± 0.7)</td>
<td>11 ± 3&lt;sup&gt;c&lt;/sup&gt; (3.1 ± 1.3)</td>
<td>Mice died by 5th wk</td>
<td></td>
</tr>
<tr>
<td>DBA-49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50 ± 2</td>
<td>25 ± 7&lt;sup&gt;c&lt;/sup&gt; (C)</td>
<td>10 ± 2&lt;sup&gt;c&lt;/sup&gt; (0.7 ± 0.2)</td>
<td>9 ± 2&lt;sup&gt;c&lt;/sup&gt; (3.1 ± 1.4)</td>
<td>Mice died by 5th wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SS</td>
<td>50 ± 2</td>
<td>12 ± 1&lt;sup&gt;c&lt;/sup&gt; (C)</td>
<td>18 ± 1&lt;sup&gt;c&lt;/sup&gt; (C)</td>
<td>10 ± 5&lt;sup&gt;c&lt;/sup&gt; (C)</td>
<td>25 ± 1&lt;sup&gt;c&lt;/sup&gt; (C)</td>
<td>70 ± 2&lt;sup&gt;c&lt;/sup&gt; (B)</td>
<td>52 ± 4 (B)</td>
<td>Tumor rejected by 5th wk</td>
<td></td>
</tr>
<tr>
<td>C77</td>
<td>50 ± 2</td>
<td>28 ± 1&lt;sup&gt;c&lt;/sup&gt; (C)</td>
<td>31 ± 11&lt;sup&gt;c&lt;/sup&gt; (C)</td>
<td>29 ± 2&lt;sup&gt;c&lt;/sup&gt; (C)</td>
<td>21 ± 7&lt;sup&gt;c&lt;/sup&gt; (0.60 ± 0.3)</td>
<td>24 ± 7&lt;sup&gt;c&lt;/sup&gt; (1.5 ± 0.9)</td>
<td>17 ± 1&lt;sup&gt;c&lt;/sup&gt; (2.9 ± 1.5)</td>
<td>Mice died by 6th wk</td>
<td></td>
</tr>
<tr>
<td>C57&lt;sup&gt;f&lt;/sup&gt;</td>
<td>SS</td>
<td>19 ± 2</td>
<td>13 ± 1&lt;sup&gt;c&lt;/sup&gt; (C)</td>
<td>42 ± 1&lt;sup&gt;c&lt;/sup&gt; (C)</td>
<td>19 ± 5 (B)</td>
<td>Tumor rejected by 2nd wk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DBA&lt;sup&gt;f&lt;/sup&gt;</td>
<td>DBA-49</td>
<td>41 ± 3</td>
<td>16 ± 3&lt;sup&gt;c&lt;/sup&gt; (C)</td>
<td>16 ± 4&lt;sup&gt;c&lt;/sup&gt; (D)</td>
<td>15 ± 5&lt;sup&gt;c&lt;/sup&gt; (D)</td>
<td>17 ± 1&lt;sup&gt;c&lt;/sup&gt; (D)</td>
<td>Mice died by 4th wk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SS</td>
<td>41 ± 3</td>
<td>14 ± 1&lt;sup&gt;c&lt;/sup&gt; (C)</td>
<td>15 ± 2&lt;sup&gt;c&lt;/sup&gt; (C)</td>
<td>27 ± 8&lt;sup&gt;c&lt;/sup&gt; (C)</td>
<td>49 ± 4 (B)</td>
<td>Tumor rejected by 4th wk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD2F&lt;sup&gt;g&lt;/sup&gt;</td>
<td>DBA-49</td>
<td>30 ± 5</td>
<td>21 ± 3&lt;sup&gt;c&lt;/sup&gt; (C)</td>
<td>19 ± 1&lt;sup&gt;c&lt;/sup&gt; (D)</td>
<td>21 ± 3&lt;sup&gt;c&lt;/sup&gt; (D)</td>
<td>8 ± 1&lt;sup&gt;c&lt;/sup&gt; (D)</td>
<td>11 ± 3&lt;sup&gt;c&lt;/sup&gt; (D)</td>
<td>Mice died by 5th wk</td>
<td></td>
</tr>
<tr>
<td>SS</td>
<td>30 ± 5</td>
<td>25 ± 4&lt;sup&gt;c&lt;/sup&gt; (C)</td>
<td>12 ± 3&lt;sup&gt;c&lt;/sup&gt; (C)</td>
<td>35 ± 4 (B)</td>
<td>32 ± 6 (B)</td>
<td>Tumor rejected by 2nd wk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L1210</td>
<td>30 ± 5</td>
<td>17 ± 2&lt;sup&gt;c&lt;/sup&gt; (D)</td>
<td>17 ± 7&lt;sup&gt;c&lt;/sup&gt; (4 days) (D)</td>
<td>11 ± 2&lt;sup&gt;c&lt;/sup&gt; (1 wk) (D)</td>
<td>17 ± 2&lt;sup&gt;c&lt;/sup&gt; (11 days) (D)</td>
<td>Mice died by 12th day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3H&lt;sup&gt;b&lt;/sup&gt;</td>
<td>SS</td>
<td>45 ± 6</td>
<td>10 ± 8 (D)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mice died by 2nd wk</td>
</tr>
</tbody>
</table>

<sup>a</sup> Six-week-old mice. Six-month-old mice had normal values of 16 ± 4 µg/liter.
<sup>b</sup> Mean ± S.D.
<sup>c</sup> p < 0.001 for difference from corresponding normal animals (Student's t test).
<sup>d</sup> Numbers in parentheses, sq cm obtained from measuring the short and long axes of the elliptically growing tumor and multiplying these 2 values to give an approximate area. Tumors with areas that could not be measured in this manner are given as: B, no palpable tumor; C, palpable but too small to measure (less than 0.5 cm on one axis); D, tumor too diffuse and disseminated to measure. These tumors grew progressively to finally cover an area estimated to be at least 4 sq cm.
<sup>e</sup> Repeat of previous experiment.
<sup>f</sup> Equal numbers of males and females approximately 3 months old.
<sup>g</sup> Males approximately 7 weeks old.
<sup>h</sup> Females approximately 7 weeks old.
no significant decrease in serum leucogenenol levels occurs after 24 hr, although the tumor progressively increases in size until the death of the animal.

Although implantation of tumor cells that will be rejected (Table 1) never leads to a measurable tumor, their implantation results in significantly lower than normal leucogenenol levels in 24 hr. In general, these levels rise above normal values at about the time the tumor is observed to be rejected. For example BALB/c and C57 mice inoculated with SS tumor cells have above normal levels of serum leucogenenol 3 and 1 week, respectively, after inoculation, that is, approximately at the time the tumor is seen to be rejected. After rejection, the serum leucogenenol levels return to normal values.

There does not appear to be a correlation of serum leucogenenol levels with the time required for the death of the animal. CD2F1 mice inoculated with DBA-49 tumor cells are alive at 5 weeks and have serum leucogenenol levels at that time that are not significantly different from those of CD2F mice that die within 12 days after inoculation with L1210 cells.

When a tumor is rejected because of the previous immunization of the mouse (Table 2), the serum leucogenenol levels are depressed during the period of tumor growth but return to normal values shortly after the tumor is observed to decrease in size.

Excision of the tumor (Table 2), which results in the recovery of the animal, is followed by the return of serum leucogenenol levels to normal values in 4 weeks. The length of time required for the serum leucogenenol levels to return to normal values suggests that the tumor interferes with leucogenenol biosynthesis.

Skin or muscle incisions or loss of blood results in a temporary elevation of serum leucogenenol (Table 10). Also, a significant decrease in serum leucogenenol levels is not characteristic of the normal immune response. Table 3 shows that a skin allograft causes an increase in serum leucogenenol levels. However, the increase does not exceed that produced by the tissue destruction associated with performing a skin graft. This makes one suspect that the elevation in serum leucogenenol levels results from tissue destruction rather than the immune response. It may well be that an elevation in serum leucogenenol levels is normally associated with the inflammatory response. In this case, the degree of elevation in serum leucogenenol levels should be associated with the degree of tissue destruction and hence not necessarily related to the size of the tumor. This could account for the apparent lack of correlation (Table 1) between the size of the tumor that is to be rejected and the time at which an increase in serum leucogenenol is observed. That the serum leucogenenol levels are actually depressed by the growing tumor suggests that the tumor liberates a substance that inhibits the release or biosynthesis of or destroys leucogenenol. That such is the case is strongly suggested by the fact (Table 4) that the s.c. injection of a cell-free 0.9% NaCl solution extract of DBA-49 tumor tissue but not of spleen tissue results in a temporary decline in serum leucogenenol levels comparable to those observed following the inoculation of viable tumor cells.

Admittedly, our results do not rule out the possibility that the depression of serum leucogenenol levels results from the activity of a virus associated with the transplanted tumors. Indeed, a virus could also be present in the 0.9% NaCl solution extract of the tumor.

However, injection of the spleen extract (no effect) or the skin allografts (elevated levels of serum leucogenenol) would also be expected to transfer viruses to the recipient animals. One would be forced to conclude that only viruses associated with tumors affected serum leucogenenol levels. Furthermore, the same tumor (e.g., SS) that grows in one strain of mouse (e.g., C3H) is rejected in another strain (e.g., BALB/c), and whether or not serum leucogenenol levels return to normal depends on whether or not the tumor is rejected. Hence, one would conclude that the antigenic activity of any virus, postulated to affect serum leucogenenol levels, is identical to that of the associated tumor. Although possible, this seems hardly likely. However, only an investigation of the effect of various viruses on serum leucogenenol levels can answer the question.

Progressive immunosuppression is frequently associated with progressive tumor growth in both laboratory animals and humans. Tumor-specific immune responses often disappear entirely (2, 6, 24, 25). Delayed hypersensitivity reactions and in vitro lymphocyte stimulation by mitogens and antigens are also depressed in many cancer patients (4, 7). The response of mouse spleen cells to both mitogens and specific antigens declines with increasing tumor size (1, 23). Recovery of an immunologically responsive state follows surgical removal of the tumor (5, 8).

Treatment with leucogenenol increases the rate at which bone marrow cells (3, 14, 19) and possibly many other types of cells develop to become mature functional cells. Possibly as a

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**Table 2**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration of leucogenenol (µg/liter) at following time after inoculation of tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated controls</td>
</tr>
<tr>
<td>Immunization</td>
<td>50 ± 4</td>
</tr>
<tr>
<td>Excision of tumor</td>
<td>50 ± 8</td>
</tr>
<tr>
<td>Sham excision of tumor</td>
<td>50 ± 4</td>
</tr>
</tbody>
</table>

* Mice approximately 7 weeks old.

b Mice immunized with DBA blood before inoculation with DBA-49 tumor. For details, see text.

c Mean ± S.D.

d p < 0.001 for difference from control animals.

<sup>e</sup> Tumors regressing.

<sup>f</sup> Tumor removed together with overlying skin. For details, see text.

<sup>g</sup> At the designated intervals of time, approximately 0.25 ml of blood was obtained by cardiac puncture from each of 12 mice. The serum was separated from the pooled blood and assayed for its concentration of leucogenenol.

<sup>h</sup> An area of skin and fascia similar in size to that removed when excising the tumor was removed from the mice on the side opposite the tumor. For details, see text.
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Table 3
Effect of skin allografts on the concentration of leucogenenol in the sera of BALB/c mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration of leucogenenol (µg/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated controls</td>
<td>1 day</td>
</tr>
<tr>
<td>Skin allografts</td>
<td>49 ± 4</td>
</tr>
<tr>
<td>Preparation of bed but no graft</td>
<td>49 ± 4</td>
</tr>
</tbody>
</table>

a Mice approximately 7 weeks old.
b Blood was obtained by cardiac puncture under anaesthesia and set aside overnight at 4°C. Then the serum was removed by a Pasteur pipet.
c A circular area of skin, approximately 2 cm in diameter, was removed from over the rib cage above the right leg, and the area was covered with a corresponding patch of skin freshly removed from a C57 mouse. For details, see text.
d p < 0.001 for difference from control animals.
e Skin grafts were intact when animals were exsanguinated.
f A circular area of skin, approximately 2 cm in diameter, was removed from over the rib cage above the right leg. For details, see text.

Table 4
Effect on the concentration of leucogenenol in the serum of BALB/c mice of giving the mice s.c. injections of a cell-free 0.9% NaCl solution extract of DBA-49 tumors

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration of leucogenenol (µg/liter) at following time after injection of extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated controls</td>
<td>1 day</td>
</tr>
<tr>
<td>Extract of tumor</td>
<td>50 ± 4</td>
</tr>
<tr>
<td>Extract of spleen</td>
<td>50 ± 4</td>
</tr>
</tbody>
</table>

a Mice were given s.c. injections of 0.05 ml of a cell-free extract prepared from a mixture of 12 g of freshly excised DBA tumors and 6 ml of 0.9% NaCl solution. Mice were also given injections of the same quantity of an extract of spleen prepared in an identical manner. For details, see text.
b p < 0.001 for difference from normal animals.

Result of its effect on the development of cells, treatment with leucogenenol enhances the immune response of animals. This is evidenced by the findings that sublethally X-radiated mice (17) and splenectomized rats (12, 13) given daily injections of leucogenenol, thus maintaining an elevated level of leucogenenol in their circulation, recover their ability to respond to the injection of sheep erythrocytes with the formation of hemolysin earlier than untreated controls. Also, normal rats (12, 13) treated with leucogenenol form peak titers of hemolysins earlier than untreated controls. Treatment of normal and splenectomized rats (15) with leucogenenol results in the formation of above normal concentrations of plaque-forming cells in their lymphoid tissues when they are challenged with sheep erythrocytes. Since above-normal concentrations of serum leucogenenol in an animal enhance the immune response of the animal, it would be expected that lower than normal concentrations of leucogenenol in the serum of the animal would be associated with a depression of its immunocompetency. This is certainly true of neonatally thymectomized mice. Such mice have a 5-fold decrease in their serum leucogenenol levels and do not reject skin allografts or form normal titers of hemolysin when challenged with sheep erythrocytes. However, treatment of neonatally thymectomized mice with leucogenenol induces them to reject skin allografts and form normal titers of hemolysin when challenged with sheep erythrocytes.4

The present studies show that tumors suppress the level of circulating leucogenenol, probably through the mediation of a substance liberated by the growing tumor. Therefore, it is suggested that the progressive immunosuppression associated with tumor growth is due, at least in part, to the suppression of serum leucogenenol levels by the growing tumor.

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