The Cytotoxic Effect of Avian Lymphoid Tumor Antiserum

B. R. Burmester, Ph. D.

(From the U. S. Regional Poultry Research Laboratory, East Lansing, Michigan)

(Received for publication January 21, 1947)

A cellular suspension prepared from a lymphoid tumor (11), when injected into the pectoral muscle of chickens, will produce a rapidly growing tumor at the injection site which will metastasize to the viscera in a high percentage of cases. Burmester, Prickett, and Belding (3) demonstrated the presence of a filtrable agent which, though incapable of producing a tumor at the site of inoculation, caused the occurrence of a high incidence of osteopetrosis and lymphoid tumors of the viscera during an experimental period of 6 months.

It was found (2) that all birds which survived takes of the first cellular implant of this tumor were invariably highly immune to a second or a third implant and that this immunity persisted for at least 202 days. Olson (12-14) treated tumor suspensions with heat, cold, chemicals, drying and centrifugation and found generally that when the growth capacity was destroyed the immunizing ability was likewise lost.

This report describes the demonstration of an antibody-like factor in the serum of lymphoid tumor immune birds and in other birds which have received repeated injections of tumor material without viable cells. The factor was identified by its in vitro toxic effect upon tumor cells and by its in vivo effect when injected into birds before and after receiving an implant of tumor cells.

I. CYTOTOXIC ACTION IN VITRO

MATERIALS AND METHODS

The general procedure used in testing the in vitro effect of antiserum was to incubate the lymphoid tumor cells in the serum to be tested, after which the mixture was injected into the pectoral muscle of 5 to 11 day-old chicks. Formation of a palpable tumor within 3 weeks at the site of inoculation was used as the measure of the viability of the tumor cells. This criterion was based on the finding of Burmester, Prickett, and Belding (3) that inocula containing tumor cells produced palpable tumors at the site of injection in 7 to 14 days; whereas cell-free extracts failed to produce tumors at the site of injection. However, chicks inoculated with such extracts developed tumors of the bone and viscera in 90 to 180 days. The avian lymphoid tumor used in this investigation was developed by Olson (11) and known at this Laboratory as strain RPL 12.

Chickens used for the propagation of the tumor, the source of serum, and the source of baby chicks for the cytotoxic tests in all experiments except numbers 10 to 15 inclusive, was an inbred line of chickens selected for its high susceptibility to naturally occurring lymphomatosis (16). Matings that supplied the chicks were maintained in strict isolation and had a low incidence of lymphomatosis (15). Chicks for Experiments 10 to 15 were the progeny of the crosses of two inbred lines developed at this Laboratory (16). All birds used in hyperimmunization were 200 or more days of age.

Lymploid tumor antiserum.—Antiserum and plasma prepared by the use of a fresh suspension of tumor cells were made in 3 different series of injections involving different groups of chickens. Series I consisted of a group of chickens that survived the original tumor cell implant and were subsequently found refactory to a second implant made 21 to 39 days later. After an interval of 148 to 220 days, the birds were given ten 1 cc. portions of fresh tumor mince injected intramuscularly at weekly intervals. Immune plasma from this series was collected 6 days after the seventh hyperimmunizing injection. The inoculum used for the repeated injections was prepared by pooling several tumors of the pectoral muscle of birds used in the serial passage of strain RPL 12. The tumor tissue was minced (10) and suspended in equal parts of Tyrode's solution.

Series II chickens were hyperimmunized a year later by essentially the same procedure used in Series I except that fewer injections of tumor mince were made prior to the collection of blood. The plasma used for testing the cytotoxic effect in the blood of birds of Series I and II was obtained by drawing blood from the heart into a syringe containing 0.1 volume heparin solution having a concentration of 0.4 gm. per 100 ml. of 0.85 per cent NaCl solution. The blood cells were sedimented by centrifugation, the plasma decanted and stored at about 2° C. until used in the tests.
In the hyperimmunization of birds in Series III, the following 5 different preparations were used:

(a) **Tumor cell suspension, unfrozen.**—Antigen "a" was prepared by mincing a composite of several intramuscular tumors from birds in the serial passage of this tumor strain. The mince was diluted with 3 parts of saline and injected into birds that had survived a previous implant of viable tumor cells.

(b) **Tumor cell suspension, frozen rapidly and thawed.**—Antigen "b" was prepared in the same manner as "a," except that, in addition, it was sealed in pyrex tubes and immersed in ethyl alcohol cooled to −76 °C by solid CO₂. After 5 minutes the inoculum was thawed in running tap water. This freezing and thawing procedure was repeated twice.

(c) **Tumor cell suspension, heated.**—Antigen "c" was prepared the same as "a," except that in addition the suspension was heated to 50°C and held at that temperature for 30 minutes in a constant temperature water bath.

(d) **Homogenized tumor suspension.**—Tumor material for this preparation, antigen "d," was obtained from the same source as for the "a" antigen. It was ground in a Waring Blender for 10 minutes with 3 parts of normal saline. The temperature of the material was maintained in the range of 2°C to 10°C by cooling at intervals in ice water mixture during the process.

(e) **Ground normal tissue suspension.**—The bursa of Fabricius, thymus, spleen, and a part of the liver from several birds raised in isolation, and which showed no gross evidence of disease, were pooled and processed in the same manner as "d." This antigen, "e," served as the normal tissue control.

Antigen "a" was injected into birds that had survived a previous implant of the same tumor strain. Preparations "b," "c," "d," and "e," were injected into male birds not previously inoculated. Four to 6 birds were used for each preparation. Injections were made every other day for 24 days. The first 2 injections consisted of 2 cc. of the antigen given intramuscularly and the remaining 10 injections of 4 cc. were given by the intraperitoneal route. No tumors developed in any of the birds injected.

Blood was collected on the sixth and 12th days after the last hyperimmunizing injection was made. The blood was allowed to clot and the serum separated with the aid of a centrifuge. The serum was sealed in glass serum tubes and stored a maximum of 213 days at 2°C until used.

**Normal serum.**—Serum was also collected from birds raised in isolation and without previous or concurrent evidence of disease. All such serum from "D" and "G" ("D" and "G" designate the chickens that were hatched during the calendar years 1942 and 1945 respectively) birds was frozen and stored at −76°C whereas all other serum was stored at 2°C until used.

**Lymphoid tumor cells.**—Cells used for testing the cytotoxic activity of the antiserum were obtained from intramuscular tumors of birds in the serial passage of strain RPL 12. The excised tumor was minced and suspended in 2 parts of saline and then filtered through 2 layers of cheese cloth. The concentration of tumor cells was estimated by the use of a standard blood cell counting chamber and appropriate dilution made to obtain the desired cell concentration before the addition of the antiserum to be tested. All tests were made by adding 9 parts by volume of antiserum to 1 part of tumor cell suspension. The total volumes used varied from 0.2 ml. to 1.0 ml. The mixture was then incubated for 2 to 24 hours at temperatures of 2°C to 37°C. The actual factors used depended upon the experiment. For most experiments antiserum from individual birds was used, but in certain cases (Table II) antiserums or plasma from several birds of the same treatment were pooled. All results reported in Tables II and III were obtained with mixtures incubated for 24 hours at 37°C. Immediately following the incubation period, 0.02 to 0.05 ml. of the mixture was injected into the right pectoral muscle of chicks of 5 to 11 days of age. The number of tumor cells per injection dose was about 1,000 for all experiments except 8 and 9, 200 cells being used for the former and 2,000 for the latter experiment. Chicks were examined for the presence of a tumor every 3 days during the period beginning with the 10th day and ending with the 19th day after inoculation. New tumors appeared very infrequently after the 16th day and only a few tumors grew after the 19th day. Since all results reported are based on the presence or absence of palpable tumors, the maximum incidence (± experimental error) was no doubt attained by the 19th day. An additional 2 days were allowed and all birds were killed and examined on the 21st day after inoculation. No lesions other than tumors were found in the inoculated chicks.

Results of inoculation are presented in the tables in terms of the fraction, that is, the number that developed tumors over the number that were inoculated. Only those with a distinct tumor growth were classified as positive. The few questionable cases that did occur were classified as negative. The activity of the tumor cell suspen-

---

1 Obtained from Central Scientific Co., Chicago, Illinois.
sion and the effect of normal serum were tested in each experiment to provide a basis for comparison with the tumor cells treated with the antiserum.

RESULTS AND DISCUSSION

The toxic effect of hyperimmune plasma on lymphoid tumor cells was detected in the first experiment, in which the cells were suspended in immune plasma for 2 hours and for 20 hours prior to their injection into young chicks. The mixture that was incubated for 2 hours produced tumors in 5 of 10 chicks injected and that incubated for 20 hours produced tumors in only 2 chicks of 9 inoculated. Tumor cells from the same source when incubated in normal plasma for 20 hours produced tumors in all of 9 birds injected.

Table I: Influence of Time and Temperature on the Cytotoxic Action of Antiserum and Plasma

<table>
<thead>
<tr>
<th>Exper. No.</th>
<th>Time (hrs.)</th>
<th>Temperature (degrees C.)</th>
<th>Lymphoid tumor antiserum</th>
<th>Normal Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1†</td>
<td>2</td>
<td>7</td>
<td>5/10</td>
<td>9/10</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>7</td>
<td>2/9</td>
<td>9/9</td>
</tr>
<tr>
<td>2†</td>
<td>2</td>
<td>2</td>
<td>9/10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>7</td>
<td>10/10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>11/11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>7/11</td>
<td>11/11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>18</td>
<td>0/10</td>
<td>10/10</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>37</td>
<td>0/10</td>
<td>9/9</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>6/7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>2/7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>3/7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>5/7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>4/7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>2/7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>2</td>
<td>3/7</td>
<td>7/7</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>20</td>
<td>1/7</td>
<td>7/7</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>0/7</td>
<td>7/7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Number that developed tumors/number inoculated.
† Plasma used.

These results suggested that plasma of birds that had survived implants of this lymphoid tumor and had received several hyperimmunizing injections, materially decreased the ability of tumor cells of the same strain to reproduce and grow into a detectable tumor in the new host. There was also some indication that the length of the incubation period of the mixture may have influenced the results.

Influence of time and temperature.—The influence of the length of the incubation period and the incubation temperature are shown in the results of 3 experiments presented in Table I. Results of the first experiment, as indicated above, showed that with an increase in the length of the incubation period from 2 to 20 hours, the tumor incidence was reduced from 5/10 to 2/9. The difference, however, is not statistically significant.

In the second experiment, incubation temperatures of 2°, 18° and 37° C. were used for periods of 2 and 24 hours. No toxic effect was obtained when the mixture was allowed to stand for only 2 hours. However, mixtures that were held for 24 hours showed a marked reduction in their tumor-producing activity. Tumor cell-immune plasma mixtures held at 2° C. for 24 hours, produced tumors in 7 of 11 chicks inoculated, but similar mixtures held at 18° and 37° C. for the same length of time produced no tumors. Tumor cells suspended in plasma from normal birds and incubated at the latter temperatures and time produced tumors in all chicks injected.

In a third experiment (No 15), tumor cells were suspended in hyperimmune serum for 3, 8, and 24 hours each at 2°, 20°, and 37° C. The results (Table I) showed that at each time period the toxic effect increased with the temperature, with the exception of the 3 hour, 20° group. This group had fewer birds with tumors than did the 3 hour, 37° group. The results also showed that within each temperature group the toxic effect increased (again with the exception of the 3 hour, 20° group) with an increase in the length of the incubation period. Thus, the cytotoxic effect increased with an increase in incubation time and temperature so that the maximum effect (no tumors in 7 birds injected) was obtained in the 24 hour, 37° C. group. This was the maximum for these factors in experiments thus far conducted. No such effect was obtained when the tumor cells were incubated in normal serum. All birds that received tumor cells incubated in normal serum for 24 hours developed tumors.

Other investigators working with other materials have used a much shorter incubation period to demonstrate a cytotoxic effect of antiserum. Kidd (8) obtained a suppression of tumor growth when the transplanted Brown-Pearce tumor cells were first incubated with antiserum for only 2 to 3 hours at 37° C. Green (7) incubated mammary cancer cells in mammary cancer milk agent antiserum for 3 hours at room temperature and then 3 hours at 7° C. and obtained a complete suppression of tumor growth.

The reason for the much longer reaction period necessary for maximal effect with the avian lymphoid tumors is not evident. Some cytotoxic effect was obtained in experiment 15 when the incubation time was only 3 hours, 2 of 7 birds developed tumors when the incubation temperature was 20° C. and the incidence was 3 of 7 when the temperature was 37° C. thus indicating that a measurable toxic effect occurs within 3 hours. That the differences may be due to a low concentration of antibodies in the serum should be
considered. Preliminary titrations indicate that the cytotoxic activity of sera thus far tested was quite low. In the first such trial (not presented in Tables) tumors grew in only 3 of 10 birds injected with tumor cells incubated in undiluted serum; whereas cells suspended in antiserum diluted 1:5 and 1:25 with normal serum, produced tumors in 8/10 and 9/10 birds, respectively. In a second test, sera from 5 different hyperimmunized birds were used. The results for the different sera were quite consistent, giving a total of 3 birds with tumors of 20 inoculated with cells suspended in the undiluted serum, and 17 with tumors of 20 implanted with cells suspended in serum diluted 1:5 with normal serum. Tumor cells suspended in normal serum again produced tumors in all birds injected. Although the incidence of tumors produced by cells suspended in diluted antiserum was high, the maximum size of the tumors and the mortality were much lower than in the normal serum group. Thus no suppression of growth was obtained with antiserum diluted 1:5. However, the effect was much less than with the undiluted antiserum. Since undiluted serum was necessary for maximal effect, then it may be inferred that a long reaction time must be used to obtain the maximal effect.

Influence of source of antiserum.—While conducting numerous tests under similar conditions but with various antisera, it was found that the apparent cytotoxic activity varied. Results of various tests in which the cells and sera were incubated for 24 hours at 20° to 37° C. are presented in Table II. Results with lymphoid tumor antiserum are from 9 different tests, using antisera from 3 different (see methods) hyperimmunizing series. Five individual samples of the antisera and 5 composite samples made by pooling the serum of sever-

<table>
<thead>
<tr>
<th>Exper. No.</th>
<th>Series</th>
<th>Source</th>
<th>Tumor incidence*</th>
<th>Source</th>
<th>Tumor incidence*</th>
<th>Source</th>
<th>Tumor incidence*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I</td>
<td>Pooled†</td>
<td>2/9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>II</td>
<td>Pooled (a) †</td>
<td>0/20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>II</td>
<td>Pooled (b)†</td>
<td>7/7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>III</td>
<td>Pooled (a)</td>
<td>0/10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>III</td>
<td>G1202H2</td>
<td>0/5</td>
<td>G1202T2</td>
<td>5/5</td>
<td>G1319U2</td>
<td>5/5</td>
</tr>
<tr>
<td>11</td>
<td>III</td>
<td>Pooled (b)</td>
<td>3/5</td>
<td></td>
<td>4/4</td>
<td></td>
<td>G1319U2</td>
</tr>
<tr>
<td>12</td>
<td>III</td>
<td>G1202H2</td>
<td>1/4</td>
<td>G1202T2</td>
<td>3/4</td>
<td>G1319U2</td>
<td>1/4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G1210J2</td>
<td>1/4</td>
<td>G1210R</td>
<td>4/4</td>
<td>D821A2</td>
<td>4/4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G1319P3</td>
<td>0/4</td>
<td></td>
<td></td>
<td></td>
<td>D805Q2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G1324Z2</td>
<td>0/4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>III</td>
<td>G1202H2</td>
<td>0/12</td>
<td>G1202T2</td>
<td>5/6</td>
<td>D821A2</td>
<td>6/6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G1319P3</td>
<td>1/12</td>
<td>G1210R</td>
<td>6/6</td>
<td>D821S</td>
<td>6/6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G1324Z2</td>
<td>4/12</td>
<td></td>
<td></td>
<td></td>
<td>D805G</td>
</tr>
<tr>
<td>14</td>
<td>III</td>
<td>G1202H2</td>
<td>0/5</td>
<td>G1202T2</td>
<td>3/5</td>
<td>D821S2</td>
<td>5/5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G1255J2</td>
<td>0/5</td>
<td>G1210R</td>
<td>4/5</td>
<td>D821K2</td>
<td>3/5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G1270J3</td>
<td>0/5</td>
<td></td>
<td></td>
<td></td>
<td>D821Z2</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>20/127</td>
<td>34/39</td>
<td>115/123</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage</td>
<td></td>
<td></td>
<td>15.7</td>
<td>27.2</td>
<td>91.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Number that developed tumors/number inoculated.
† Plasma used.
(a) (b) See description under Methods.

Sera of 2 birds (G1202T2, G1210R in Table II) that had received repeated injections of minced normal lymphoid tissue were tested for their cytotoxic effect with the same lymphoid tumor cell suspensions used with the lymphoid tumor antiserum. In the 7 different tests made, the incidence of source of antiserum varied from a low incidence of tumors of 0/20 for composite “a” of hyperimmunizing series II, to a high of 7/7 for composite “b” of series II. A low incidence of no tumors occurred in most of the tests conducted. In almost one-half of the tests (9 of 17) none of the birds developed any gross evidence of tumors. Of a total of 127 birds used in testing the cytotoxic activity of the 17 different sera, only 20 birds (15.7 per cent) developed tumors and over one-third (7) were from the one high incidence test cited above (composite “b,” series II).

Sera of 2 birds (G1202T2, G1210R in Table II) that had received repeated injections of minced normal lymphoid tissue were tested for their cytotoxic effect with the same lymphoid tumor cell suspensions used with the lymphoid tumor antiserum. In the 7 different tests made, the incidence...
of tumors varied from 3/5 to 6/6, giving an average of 87.2 per cent for the 39 birds used in the 7 tests.

Lymphoid tumor cells used in testing the cytotoxic activity of the various antisera were in each test also suspended in serum from normal birds, prior to their implantation, in order to test the growth activity of the tumor cells and the effect of the incubation period and temperature. The sera from 14 different normal birds were used in 19 different tests. A 100 per cent incidence of tumors was obtained in all except 3 tests and 2 of these were with the same serum samples (G1319U2). However, a 100 per cent incidence was obtained in 2 other tests, using the same serum. Thus, a considerable variation in results was obtained in several tests of the same normal serum. This error in the determination is no doubt due in part to the unrecognized variation in the test animals, material and technics. It should also be recognized that birds that were used as the source of normal serum or antiserum may have carried a low titer of similar antibody-like factors, i.e., they may have had lymphomatosis in an early or inapparent form.

Duran-Reynals (5) found that antibody-like factors endowed with the property of suppressing the effect of viruses of Rous and Fuginami sarcoma develop in the blood of normal fowls paralleling the growth of the individual. No indication of a phenomenon of this sort was found in working with materials and birds reported herein. Untreated birds having band numbers with the prefix “D” supplied the normal serum when they were 300 days of age. Birds with the prefix “H” supplied the normal serum when 60 to 110 days of age. In a test, the results of which are not given in Table II, serum from untreated birds 16 days of age was used. Results with serum of birds of all age groups were similar in that no cytotoxic effect was obtained, indicating that the antibody-like factor of Duran-Reynals, which is present in normal adult fowls, does not appear to play a part in the suspension of the growth of lymphoid tumors herein described.

Results presented in Table II suggest that there is some variation in the cytotoxic activity of various lymphoid tumor antisera. The variation in results obtained in several determinations of the same serum, indicate that some of these variations may be due to errors inherent in the method.

In testing Tyrode’s solution as a possible diluent for tumor cells and antisera, it was found that when the tumor cells were suspended in Tyrode’s solution for only 2 hours at 20°C, some growth activity resulted. When the holding period was increased to 24 hours the growth activity was completely lost in two such tests; however, when as little as 10 per cent by volume of normal serum was added to the Tyrode’s solution, the tumor cells retained their full tumor inducing activity after 24 hours at 20°C.

**Influence of heating the antiserum.**—Sera from 2 hyperimmunized birds were heated to 56°C, 61°C and 66°C for 30 minutes by placing 2 cc. portions of the serum in small test tubes and then immersing the lower half of each tube in a constant temperature water bath, the fluctuation of which was not more than 0.5°C. After cooling again to room temperature, lymphoid tumor cells were added to the various tubes of heated and unheated antiserum and to a tube of unheated normal control serum. After each mixture was incubated at 37°C for 24 hours, it was injected into 7 chicks. The results obtained gave no indication of a decrease in cytotoxic activity even at the highest temperature used (66°C). No tumors developed in any of the birds that received cells suspended in heated or unheated antiserum, whereas all of 7 birds inoculated with tumor cells suspended in normal serum developed tumors.

**Influence of using killed or disintegrated tumor cells on the activity of the antiserum.**—Chickens injected with antigens “b,” “c,” and “d,” as described in the section on methods and materials, had received no previous injection of cellular or cell-free lymphoid tumor material and they did not develop tumors after injection of the antigens. It can, therefore, be assumed that antigens “b,” “c,” and “d” were relatively free of viable tumor cells, since it is known that relatively few viable tumor cells are necessary for the initiation and growth of this tumor in chickens.

The antigens containing killed or disrupted tumor cells produced no visible reaction in the birds receiving the several injections during the hyperimmunization period. However, results of 4 experiments testing the cytotoxic activity of antisera produced with killed cells, indicated that an antibody-like factor was present in the sera, similar to that found in birds which had received viable tumor cells.

Results of these experiments are presented in Table III. Tumor cells incubated in antiserum against rapidly frozen and thawed cells, produced no tumors in 4 tests and did produce tumors in 2 to 3 of the birds in 3 other tests, making a total of 6 positive out of 35 injected. Tumor cells incubated with antiserum against heated cells, produced no tumors in 2 tests. Tumor cells suspended in antiserum against tumor material which had been homogenized in a Waring Blender, caused no tumors in 6 tests with 28 chicks. That all cell sus-
pensions used in testing the sera were highly active in all tests is indicated by the 100 per cent incidence obtained when the suspensions were incubated with normal serum. These results indicate that the cytotoxic activity of the serum was not dependent upon a viable and functional cell.

The foregoing experiments show conclusively that an antibody-like factor may be produced in the serum of birds which inhibits partially or completely the growth of the cells of this avian lymphoid tumor. This effect was obtained with serum of birds surviving several implants of viable tumor cells, as well as with serum from birds receiving several injections of tumor cells killed by freezing and thawing, by heat, and by homogenization. Thus, it would appear that the antigenic unit was a constituent of the tumor cell.

The failure to produce a similar antibody by the repeated injection of lymphoid tissue from normal birds prepared in a manner similar to the lymphoid tumor preparation would indicate that isoantigens were not involved. Since the antigenic material came from the same inbred line as the hyperimmunized birds antigenic differences between individuals were presumably much less than if unrelated individuals had been used.

The cytotoxic effects obtained with this avian lymphoid tumor are similar to the results reported for 2 mammalian tumors. Green (7) prevented completely the growth of transplanted mouse mammary cancer by mixing the tumor cells with its antiserum 6 hours before implantation. Normal mammary antiserum and normal rabbit serum produced only a slight inhibition of growth. A similar antiserum was found to neutralize the mouse mammary-tumor agent (1, 6). A suppression of growth of Brown-Pearce tumor cells by a specific antibody in vitro and in vivo was demonstrated by Kidd (8, 9). Such antibodies were found in the serum of rabbits in which an implanted tumor had regressed and in some rabbits that had received several injections of saline extracts of the tumor. Antiserum which was heated to inactivate the complement retained its antiproliferative effect.

Although it is known that extracts of lymphoid tumor will induce tumors in the viscera (3), experiments have thus far not been conducted to test the relationship between this filtrable tumor-inducing agent and the antigenic agent described herein.

II. IN VIVO EXPERIMENTS

PROCEDURE AND RESULTS

General.—The procedure employed for testing the in vivo effect of hyperimmune plasma was to make repeated injections into young chicks that had received implants of lymphoid tumor cells.

The chicks used in Experiments 1 and 2, and for one group of Experiment 3 (Stock A) were from the same lymphomatosis-susceptible, yet relatively disease-free, line of chickens which supplied chicks for all in vitro experiments with the exception of numbers 10 to 15. Stock B chicks used for 2 other groups of Experiment 3 were obtained from several matings of birds from lines susceptible to lymphomatosis (16). The birds used in these matings were classified as contaminated stock because a relatively high percentage of lymphomatosis occurred naturally in their progeny. The hyperimmune plasma used for the injections was collected from the series I group described in the previous section.

The presence of a tumor at the site of inoculation was determined by palpation at 3 day intervals and by examination at autopsy if the bird died.

Experiment 1.—Thirty-one chicks 7 days of age received 200 lymphoid tumor cells (RPL 12) by injection into the deep pectoral muscle. Six of these birds did not receive plasma injections. The remaining 25 chicks were divided into 5 groups and received daily injections of 2 ml. of hyperimmune plasma. The route and number of injections before and after implantation of the tumor cells varied with each group. Five birds that did not receive implants of tumor cells were also given the maximum number of plasma injections. The treatment of each group and the results obtained are presented in Table IV. The longest plasma injection period was 2 days before to 10 days after implantation inclusive: the shortest, 8 days after to 10 days after implantation inclusive. The incidence of tumors among the five groups during
the 28 day post-tumor implantation period varied from 5/5 to 3/5 and the survival from 3/5 to 4/5. Neither measure showed any particular relation to the number of hyperimmune plasma injections given. The 6 birds that received no plasma died with tumors and the 5 birds that were not injected with tumor cells, but received the maximum number of plasma injections, survived and showed no evidence of tumors.

<table>
<thead>
<tr>
<th></th>
<th>Number injected</th>
<th>Number with tumors</th>
<th>Number surviving</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma injections before (−) and after (+) tumor cells implanted</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperimmune plasma only†</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Hyperimmune plasma −2 to +10</td>
<td>5</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Hyperimmune plasma +1 to +10</td>
<td>5</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Hyperimmune plasma +5 to +10</td>
<td>5</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Hyperimmune plasma +5* to +10*</td>
<td>5</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Hyperimmune plasma +8 to +10</td>
<td>5</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>No plasma injected</td>
<td>6</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

Probability, † Plasma vs. no plasma = .45 .02

**Experiment 1 (Stock A):**

- Normal plasma 0 to +14 10 1 10
- Normal plasma +6 to +14 10 7 7
- Probability, † immune vs. normal plasma = .01 .01

**Experiment 2 (Stock A):**

- Stock A, hyperimmune plasma +5 to +11*, +13* 68 67 28
- Stock B, hyperimmune plasma +6 to +12*, +14* 61 51 23
- Stock B, no plasma injected 15 13 2
- Probability, † immune plasma vs. no plasma = .20 .16

* By intravenous route—all other injections by intraperitoneal route.
† Plasma injections over same period as group below.
‡ Chi square test.

This preliminary experiment indicated that the hyperimmune plasma had a definite effect on the growth and malignancy of transplanted tumors. Though the effect on the incidence of tumors may be questionable, the difference in mortality between the birds treated with plasma and those without such treatment was quite pronounced.

**Experiment 2.—**In this experiment 30 chicks 3 days of age were inoculated with 1,000 lymphoid tumor cells. Ten of the chicks were injected intraperitoneally with hyperimmune plasma in doses of 1 ml at 0, 2, and 4 days after inoculation and 2 ml at 6, 8, 10, 12, and 14 days after inoculation. The second group of 10 inoculated chicks was given 2 ml of the same plasma on alternate days beginning with the sixth and ending on the 14th day after inoculation. The remaining 10 chicks were injected with plasma collected from adult birds that had previously received no injections and that had showed no pathological lesions. During the experimental period of 21 days, only 1 bird of the 10 that had received immune plasma during the 14 day period developed a tumor and all birds survived; whereas among those that received normal plasma during the same period, 8 birds developed tumors and only 2 survived (Table IV). The majority of chicks, receiving the immune plasma during the 6 to 14 day period developed tumors but most of these also survived. These results confirm those obtained in the first experiment suggesting that the hyperimmune plasma reduced materially the malignancy of the tumor. In addition, there was some indication that the actual incidence of tumors was reduced when the injections began early.

**Experiment 3.—**The primary purpose of the implants and injections made in chicks listed under Experiment 3 was to produce a number of strain RPL 12 tumor-immune chickens to be used in connection with another phase of this study already reported (4). At 2 days of age all chicks received 500 tumor cells, and at 38 days, 500,000 tumor cells. As a measure to reduce the mortality from these implants, 1.0 cc. of hyperimmune plasma was injected daily from the fifth and sixth to the 13th and 14th days after tumor implantation for stocks A and B, respectively. Fifteen chicks of stock B were also implanted with tumor cells but did not receive any plasma injections and thus served as controls on the effect of the plasma treatment.

During a period of 57 days after the first implantations most of the birds developed tumors at the site of inoculation. The plasma had very little effect on the incidence of tumors, since 83 per cent of the stock B plasma-treated birds, 99 per cent of the stock A birds, and 100 per cent of the birds that did not receive plasma, developed tumors; however, its apparent effect in reducing the malignancy of the tumors was much greater. The rate of survival of the plasma-injected groups was 41 per cent for stock A and 38 per cent for stock B, whereas only 13 per cent of the controls survived.
DISCUSSION

A considerable reduction in the malignancy of tumor transplants, as the results of injection of hyperimmune plasma, was consistently obtained in the three experiments. The effect of the plasma on the growth of the tumor was not so consistent. Results of Experiment I indicate that the immune plasma caused some reduction in the incidence of primary tumors although no relation to the number of injections given is apparent. However, in Experiment 2 a reduction in the incidence of tumors was quite pronounced in the group that received immune plasma injections beginning with the day of implantation.

For the 3 experiments the average incidence of tumors in the chicks that received no plasma, or were injected with normal plasma, was 94 per cent. The over-all average for those that received the hyperimmune plasma was 63 per cent. Of those that received immune plasma injections beginning within 1 day after tumor implantation, 45 per cent developed a tumor. The results thus indicate that not only was there a reduction in mortality among tumor implanted birds as the result of injections of hyperimmune plasma, but there was also a marked reduction in the tumor incidence.

SUMMARY

I. The cytoxic activity of serum from normal, non-injected chickens and others that had received several injections of lymphoid tumor antigen and normal lymphoid tissue antigen was investigated in a series of 11 experiments.

(a) When lymphoid tumor cells were suspended in normal serum for 24 hours at 20° to 37° C. and then injected into the pectoral muscle, nearly all (93.5 per cent) of the 123 young chicks developed tumors. However, when lymphoid tumor cell antiserum was used instead of the normal serum, only a few (15.7 per cent) of the 127 chicks developed tumors.

(b) The cytoxic activity of the lymphoid tumor cell antiserum was considerably less when the incubation temperature was reduced to 2° to 7° C. or the incubation period to 2, 3, and 8 hours.

(c) Antiserum produced by the repeated injections of lymphoid tumor cells that had been killed or broken up by rapid freezing and thawing, homogenizing, or heating to 50° C., was also found to be highly toxic to lymphoid tumor cells.

(d) Serum from chickens that had received repeated injections of lymphoid tissue from normal birds had no effect on the growth of lymphoid tumor cells.

(e) Heating lymphoid tumor antiserum to 56°, 61°, or 66° C. for 30 minutes appeared to have no influence on its cytoxic activity.

The results suggest that an antigen, absent in normal lymphoid tissue but present in the lymphoid tumor cell, provokes the formation of an antibody-like factor in normal adult chickens which is toxic to the tumor cells. This toxicity is expressed by rendering most tumor cells nonviable after an incubation period of 24 hours and suppressing the rate of growth and malignancy of cells that do survive.

II. The effect of lymphoid tumor antiserum (plasma) injections upon the suppression of growth and malignancy of lymphoid tumors implanted in young chicks was studied in three experiments involving 220 birds.

(a) It was found that when plasma from birds hyperimmunized against a lymphoid tumor was injected into birds bearing implants of the same tumor strain, the resulting mortality was much lower than when no plasma was injected or when plasma from normal birds was used.

(b) The incidence of tumor takes was lower when the hosts received injections of hyperimmune plasma.

In summary it may be said that a factor, or factors, found in this lymphoid tumor and antigenically different from constituents of normal lymphoid tissue, is capable of provoking the production of an antibody-like factor in chickens of the same inbred line and that this factor is toxic to tumor cells of the same strain. The effect of this factor is manifested by a partial or complete suppression of the growth of tumor implants. When antiserum is injected into birds with implants, a reduction in mortality and incidence of tumors may be expected.

REFERENCES

The Cytotoxic Effect of Avian Lymphoid Tumor Antiserum

B. R. Burmester


Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/7/7/459.citation

Sign up to receive free email-alerts related to this article or journal.

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.