EXPERIMENTS ON THE RÔLE OF LYMPHOID TISSUE IN THE RESISTANCE TO EXPERIMENTAL TUBERCULOSIS IN MICE.

II. EFFECT OF CANCER IMMUNITY ON RESISTANCE TO TUBERCULOSIS.

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(Received for publication, January 20, 1917.)

An investigation recently reported from this laboratory seems to bear out the conception that the lymphocyte plays a part in the resistance of the animal to tuberculous infection.1 This work had for its starting-point the observation of Lewis and Margot2 that mice experimentally infected with bovine tubercle bacilli developed splenic enlargement. Lewis and Margot also showed that animals splenectomized about 3 weeks before the injection of the tubercle bacilli exhibited greater resistance to the infection than did intact animals. In a study in this laboratory of blood changes after splenectomy, it was observed that the majority of mice so treated developed a marked lymphocytosis by the 19th to the 21st day after the operation.3 It was thought probable, therefore, that this lymphocytosis might be a factor in causing the greater resistance displayed by the splenectomized animals. The following experiments confirm this view. Mice splenectomized and then exposed to repeated small doses of x-ray, which had been demonstrated to affect primarily the lymphoid

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1 Murphy, Jas. B., and Ellis, A. W. M., J. Exp. Med., 1914, xx, 397.
3 L. B. Lange and Jas. B. Murphy, in an unpublished study of the blood of fifty mice before, and at intervals after splenectomy, showed that immediately after the operation there was a fall in the total lymphoid count. But by the 19th to the 21st day, 75 per cent of the animals counted showed an average increase of 11,000 lymphocytes per c.mm., or a gain of over 100 per cent above their normal count. The 25 per cent which did not gain averaged a loss of about 8 per cent of their former count.
organs, proved to be more susceptible instead of more resistant to infection than were either normal animals or animals splenectomized alone (Text-fig. 1). Intact x-rayed mice were likewise highly susceptible to infection with the bovine tubercle bacilli. Morton has observed also that the x-rayed guinea pig is more susceptible to infection with the human type of the tubercle bacillus than is the normal animal.

![Text-fig. 1](image-url)

**Text-fig. 1.** Each horizontal line represents the time of survival of a mouse after infection with bovine tubercle bacilli. The first group received a series of small doses of x-ray before inoculation, and the average duration of life was 7 days. The second group was normal and averaged 26.4 days of life after inoculation.

*The Use of Cancer Immunity as a Method for Producing Lymphocytosis.*

As this evidence is of an indirect nature, it seemed desirable to obtain more direct data bearing on this conception. The present communication deals with the influence of a pronounced lymphocytosis induced by other means than splenectomy on the resistance of the mouse to tuberculous infection. We have already shown that mice immunized against and then inoculated with one of the transplantable mouse cancers developed a marked lymphoid reaction in the blood which lasted several weeks. This method, therefore, affords a convenient way of obtaining what may be regarded as non-specific high blood lymphocytic reaction which lasts long enough to answer the experimental requirement.

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Experiment 1.—Twenty mice were injected subcutaneously with 0.3 cc. of defibrinated mouse blood. These, with twenty control animals, were inoculated 10 days later with a transplantable mouse carcinoma. At the end of 3 weeks 85 per cent of the control mice had developed tumors, while only one of the surviving sixteen immunized animals showed tumor. The fifteen immune animals, with ten normal mice from the same lot were each injected intraperitoneally with 3 mg. of a 9 day culture of bovine tubercle bacilli from a glycerol agar slant, taken up in 0.5 cc. of normal salt solution. The mice were placed in individual jars in order to prevent loss from epidemic disease. Autopsies were performed as soon after death as possible, and films were taken from the peritoneal fluid, kidneys, liver, spleen, lungs, and heart's blood. The gross and micropathology will not be described at this time. The distribution of the microorganisms is shown in Table I. The average survival after inoculation for the control animals was 20.3 days, while that of the cancer immune mice was 47.7 days; that is, they lived more than twice as long. The rate at which the mice died is shown in Text-fig. 2.

### TABLE I.

**Distribution of Tubercle Bacilli in the Cancer Immune Mice and the Control Mice from Experiment 1.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Average time of survival</th>
<th>Percentage of animals with tubercle bacilli in various organs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>days</td>
<td>Exudate</td>
</tr>
<tr>
<td>Controls</td>
<td>20.3</td>
<td>100</td>
</tr>
<tr>
<td>Cancer immune mice</td>
<td>47.7</td>
<td>86.6</td>
</tr>
</tbody>
</table>

Experiment 2.—Nineteen mice were injected with an immunizing dose of 0.3 cc. of defibrinated mouse blood. 10 days later these animals, with ten controls, were inoculated with a transplantable carcinoma. After 3 weeks, four of the nineteen immunized animals and nine of the controls had developed tumors. At this time all the immunized animals, including the four which had developed tumors, with ten normal mice as controls, were each inoculated intraperitoneally with 3 mg. of a 27 day old culture of bovine tubercle bacilli\(^7\) taken up in 0.5 cc. of normal salt solution. The mice were kept throughout the experiment in individual jars. The distribution of the organisms at autopsy is shown in Table II. The average duration of life after inoculation for the control animals was 14.7 days, and for the cancer immune animals 24.3 days. The four animals which had been immunized but developed tumors lived on an average of 17.7 days. The rate at which the mice died is shown in Text-fig. 3.

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\(^7\) The same culture was used in this experiment as in the first, but in the interval it had been passed through an x-rayed guinea pig.
TEXT-FIG. 2. The horizontal lines show duration of life after inoculation of normal and cancer immune mice with tubercle bacilli. The average time of survival of the normal animals was 20.3 days, and of the cancer immune animals 47.7 days.
TABLE II.

Distribution of Tubercle Bacilli in the Cancer Immune Mice and the Control Mice from Experiment 2.

<table>
<thead>
<tr>
<th>Group</th>
<th>Average time of survival</th>
<th>Percentage of animals with tubercle bacilli in various organs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>14.7</td>
<td>100</td>
</tr>
<tr>
<td>Cancer immune mice</td>
<td>24.3</td>
<td>100</td>
</tr>
</tbody>
</table>

TEXT-Fig. 3. The lines show the survival of individual mice after inoculation with tubercle bacilli. The normal animals averaged 14.7 days, while the cancer immune animals averaged 24.3 days.

The two experiments agree in showing that mice first immunized to, and then inoculated with a transplantable mouse cancer exhibit a greater resistance to bovine tuberculosis than do normal animals. On the other hand, we have noticed that while animals ineffectively immunized against cancer show a definite lymphoid reaction in the blood, it is less marked than when the immunity is perfect. In this connection, it is of interest to observe that the four mice of this kind in Experiment 2 outlived the controls but succumbed earlier than the completely immune ones. However, a larger series of animals would be needed to establish this point.

Destruction of Lymphocytes in Cancer Immune Mice and Resistance to Tuberculous Infection.

If the lymphocytic reaction in the cancer immune animals is the factor which determines the enhanced resistance to tuberculous
infection, the destruction of these cells by means of the x-ray should reduce resistance to a point even below that of normal animals. The following experiment was performed with this in view.

Experiment 3.—Thirty-eight mice were immunized with 0.3 cc. of defibrinated mouse blood, and 10 days later, with ten control animals, were inoculated with a transplantable mouse carcinoma. Five of the thirty-eight immunized animals developed cancers, as did all the controls. 2 weeks after the cancer inoculation, eighteen of the immune animals were given a daily dose of x-ray until they had received seven exposures. At this time, or 3 weeks after the cancer inoculation, both the x-rayed and non-x-rayed cancer immune mice were each inoculated intraperitoneally with 2 mg. of a culture of bovine tubercle bacilli. The same observations were made as in the two previous experiments. The distribution of the microorganisms is shown in Table III. The average duration of life after the tubercle bacilli inoculation in the x-rayed cancer immune animals was 14.5 days and in the non-x-rayed cancer immune animals 32.5 days (Text-fig. 4).

<table>
<thead>
<tr>
<th>Group</th>
<th>Average time of survival</th>
<th>Percentage of animals with tubercle bacilli in various organs</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-rayed cancer immune mice</td>
<td>days 14.5</td>
<td>Exudate 100, Spleen 94.2, Liver 77.7, Heart's blood 5.5, Lungs 33.3, Kidneys 55.5</td>
</tr>
<tr>
<td>Intact cancer immune mice</td>
<td>days 32.5</td>
<td>Exudate 95.2, Spleen 80.9, Liver 66.6, Heart's blood 4.7, Lungs 28.5, Kidneys 33.3</td>
</tr>
</tbody>
</table>

Experiment 4.—Ten mice, which had been proved immune to cancer as a result of an injection of defibrinated blood, were given a series of doses of x-ray sufficient to reduce their lymphoid tissue. 3 weeks after the cancer inoculation, and 24 hours after the last x-ray exposure, the ten mice, with ten normals, were injected intraperitoneally with 3 mg. of a 3 weeks old culture of bovine tubercle bacilli taken up in 0.5 cc. of normal salt solution. All the animals at this time were in excellent physical condition. The same general routine was observed as in the former experiments. The x-rayed cancer immune animals died off very rapidly, the average duration of life being 8.9 days. The control mice died at a much slower rate and averaged 18 days of life after the inoculation; that is, they lived twice as long as the x-rayed series (Text-fig. 5).
TEXT-FIG. 4. The average duration of life of cancer immune mice subjected to repeated small doses of x-ray before inoculation with tubercle bacilli compared with the duration of life of intact cancer immune animals inoculated with the same infecting agent.

TEXT-FIG. 5. The average duration of life of cancer immune animals subjected to repeated small doses of x-ray before inoculation with tubercle bacilli compared with the duration of life of normal mice inoculated with the same infecting agent.

These experiments demonstrate conclusively that the enhanced resistance of cancer immune mice to tuberculous infection can be set aside or even reduced to a state of increased susceptibility by destroying the lymphocytes by means of exposure to x-rays.

DISCUSSION.

The experiments reported were not undertaken with the idea of establishing a relationship between cancer and tuberculosis, as we know of no sufficient reason to assume the existence of such a relationship. However, it has long been believed that some such specific antagonism between the two conditions exists. But considering that the ages at which cancer and tuberculosis reach their highest incidence are widely divergent, and that cancer rarely, if ever, attacks a debilitated individual, this idea of a specific antagonism would seem to have little basis in fact. That the two diseases may occur simultaneously in the same individual is borne out by many reports made in recent years.

It is probable that the lymphoid tissue of the body may be a determining factor in the phenomena of resistance in both tuberculosis and cancer, and yet the modus operandi involved may be absolutely different. Indications that the lymphoid cell plays a part in resistance to tuberculosis are numerous. The constant association of
these cells with the lesions of the disease is so well recognized that it need not be gone into here. In most of the clinical blood studies of tuberculous individuals, the white cells have been analyzed from the point of view of the polynuclear cell, but as these do not increase markedly, little attention has been paid to the other white cells. However, the blood counts show an interesting variation in the lymphoid cells which has not attracted the attention it merits. The total number of lymphocytes per c.m.m. of blood in a normal adult varies between 1,500 and 2,500 cells. In one case of advanced pulmonary tuberculosis reported by Emerson, there were only 530 lymphocytes, and in a case of miliary tuberculosis studied by the same author, the total number was only 227. Warthin reports a case of miliary tuberculosis in which the lymphocytes made up only 5.5 per cent of a subnormal white cell count. On the other hand, in cases which give a good prognosis, these cells may form 40 to 50 per cent of a white cell count somewhat higher than normal, which means a substantial increase in the lymphoid cells. Wack has observed that cases of tuberculosis advancing rapidly show a decrease, and those healing or healed a corresponding increase in the mononuclear cells. While the reports in the literature are not as convincing as might be desired, yet they present indications to the effect that the lymphocytes vary directly as the degree of resistance to the tuberculous infection. To bring together the evidence which bears on the participation of the lymphocyte in resistance to tuberculous infections, we have (a) its constant association with the pathologic lesions of the disease, (b) its fluctuation in the blood with the progress of the infective process, (c) the reduced resistance to the infection in animals depleted of their lymphoid tissue by means of x-ray, and (d) a marked enhancement of resistance to the infection displayed by animals with a high lymphocyte count resulting from the establishment of cancer immunity.

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SUMMARY.

Mice so x-rayed as greatly to reduce the lymphoid tissue are rendered highly susceptible to tuberculous infection. On the other hand, when a marked lymphocytosis is induced by first immunizing mice against, and then inoculating them with cancer, the resistance to tuberculous infection is greatly enhanced. This heightened resistance may be set aside and even changed to a state of increased susceptibility to the infection by again depleting the lymphocytes by means of the x-ray.