The Effect of the Supply of Oral Antibiotic on the Fecal Flora and Mortality of Mouse Radiation Chimeras

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Radiation chimeras, i.e., animals that received and retained allogeneic or xenogeneic hemopoietic cells after lethal whole-body irradiation, are very sensitive to bacterial invasion [1] and suffer frequently from infections with gram-negative bacteria of endogenous origin [2]. In germfree chimeras, the infectious complications are absent, and this reduces the severity of the so-called secondary disease that develops due to immunologic incompatibility between grafted cells and their host [3]. Treatment with antibiotics can also suppress the secondary disease [4].

The present paper deals with some observations on the effect of treatment with different antibiotics and combinations on the survival and bacteriologic status of chimeric mice receiving immunosuppressive treatment. This situation was encountered in a study concerned with experimental treatment of a transplanted murine leukemia. In short, leukemic mice were irradiated and injected with allogeneic spleen cells for eradication of the leukemia, and received thereafter immunosuppressive treatment in an attempt to terminate the activity of allogeneic cells and thus prevent death from the acute secondary disease [5]. The first experiments, performed in conventional animals, involved high mortality during immunosuppression, with a high incidence of bacteremia caused by Proteus mirabilis and Escherichia coli. In order to make further experimentation fruitful, we felt it necessary to attempt to eliminate the complications resulting from endogenous infections with these intestinal bacteria. We tried to reduce the bacterial flora of the digestive tracts of the chimeras by using 2 different schedules of treatment with antibiotics. The first regimen was aimed at "complete decontamination," i.e., at elimination of all bacteria that could be cultivated from the feces under standard aerobic conditions [6]. The other treatment schedule was designed to eliminate "selectively" the Enterobacteriaceae, Proteus, and E. coli [7].

Materials and Methods

Mice. Inbred female RF/O mice (H-2k), 3–4 months old, were inoculated with 10⁶ cells of a transplantable myeloid leukemia, irradiated 4 days later with 530 rad of X-ray, and then injected with 10–50 × 10⁶ spleen cells and 40–60 × 10⁶ bone marrow cells obtained from inbred C57BL/Rij (H-2b) mice (see [5] for details). Dead mice were autopsied and their cardiac blood was cultured for bacteriologic determination.

Housing of mice. The mice were housed 2–3 per cage in polycarbonate cages (12 × 13 × 18 cm) kept about 20 cm apart in a laboratory rack, which was closed with sheets of transparent polyvinyl chloride [6] and sprayed with 2% peracetic acid at weekly intervals. The cages, bedding material (sawdust), drinking bottles, and food were replaced 2–3 times a week by autoclaved material. The cages were handled with rubber gloves disinfected with 70% alcohol, and the mice were handled with sterile surgical gloves or a sterile forceps. All of these precautions in housing and

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1 Allogeneic = from another strain; xenogeneic = from another species; syngeneic = from the same strain.
handling will be called “barrier nursing.” During irradiation the mice were kept in a sterilized circular box made of plastic material.

**Antibiotic regimens.** The antibiotics (all Mycopharm, Delft) were administered via the drinking water: streptomycin 6 mg/ml, kanamycin and neomycin 4 mg/ml, and bacitracin 2 mg/ml. Mice consumed approximately 4 ml of water per day.

The antibiotic mixtures (streptomycin + bacitracin, kanamycin + bacitracin, or neomycin + bacitracin) given to eliminate whole aerobic flora from the feces were chosen by a sensitivity test [6]. Starting 10–14 days before irradiation, this “combined antibiotic treatment” was continued until the animals died (usually 8–20 days after irradiation) or until they became colonized with bacteria resistant to any of the mixtures used. The supply of antibiotics was then replaced with acidified water (pH 3), but the barrier nursing was continued as long as the animals lived.

For selective elimination, *Proteus* and *E. coli* were isolated from the feces and tested with streptomycin, kanamycin, or neomycin. The “single antibiotic regimen” was started immediately after irradiation and continued (a) until death of the animals (days 8–20), (b) until colonization with Enterobacteriaceae resistant to any of the 3 antibiotics used (this took place only exceptionally), or (c) until the clinical condition of the animals definitively improved, which improvement usually occurred about day 30. When the antibiotic supply was discontinued, the mice were supplied with acidified drinking water and barrier-nursed until 100 days after irradiation. The survivors were then transferred to the “conventional” experimental mouse room.

**Culturing feces.** Fresh fecal pellets were collected at 7–10-day intervals during antibiotic treatment for an investigation of the presence of bacteria in mice receiving the combined antibiotic regimen, and the presence of Enterobacteriaceae in mice receiving the single antibiotic regimen. In the first case, the feces from each cage were cultured in brain-heart infusion broth (Oxoid) and in Brewer’s semisolid thioglycollate (Difco) at 37 C. In case growth was observed, subcultures were made on blood agar plates for aerobic and anaerobic culturing. In the second group, the feces were first inoculated into the brain-heart infusion broth, and after 24 hr of incubation at 37 C they were subinoculated on endoagar (Difco) for another incubation period of 1 day at 37 C.

If during treatment with antibiotics the animals became positive for a bacterial species, the sensitivity pattern toward the antibiotics was reinvestigated and the antibiotic supply was changed (or continued) accordingly. The feces were cultured in the same manner for the sensitivity test before the antibiotic treatment.

**Cardiac blood.** Under aseptic conditions the thorax was opened at autopsy. An incision was made in the heart, the blood was sampled with a loop (0.02 ml) and then streaked onto blood agar for aerobic incubation at 37 C.

**Treatment of the acute secondary disease.** Cyclophosphamide (Endoxan, Asta-Werke, Germany) was injected intraperitoneally every day from day 4 to day 15 or 16 after irradiation in a total amount of 425–450 mg/kg. Other mice were X-irradiated again with 300 rad on day 9 or 21, or received injections of specific anti-donor alloimmune serum on days 4–6, 6–8, or 6–17, in a total amount of 0.3–4.0 ml per mouse. This immunosuppressive treatment was then followed by several (usually 3–4) injections of syngeneic hematopoietic cells and blood, in an attempt to terminate the chimeric state and to prevent the chronic secondary disease, or the immunosuppression was continued by weekly injections of antilymphocyte serum in a total amount of 8–9 ml per mouse. This treatment has been described in detail elsewhere [5, 8, 9].

**Statistical methods.** Data were evaluated by the chi-square test (3 × 2 or 2 × 2 table method) or by Fisher’s exact probability test. Values of \( P \) below the 5% level were accepted as showing significant difference between the data compared.

**Results**

Mice supplied with streptomycin, kanamycin, or neomycin in the drinking water usually remained free of *Proteus* and *E. coli* as long as they lived or the experiment lasted. On the other hand, the 3 antibiotics combined with bacitracin failed to keep the feces “decontaminated” (free of flora detectable by the methods used) for longer than 2–3 weeks. The animals usually became contaminated with *Enterobacter cloacae* or *Klebsiella*.

Table 1 shows the incidence of terminal bacteremia in chimeras receiving either of the 2
Table 1. The effect of antibiotic supply on the incidence of different bacterial species in the cardiac blood of animals dying with bacteremia after irradiation, injection of C57BL spleen cells, and various procedures for suppression of the graft-versus-host reaction. (A few cultures were mixed.)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Chimeras</th>
<th>Organisms isolated in cardiac blood</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total no. treated</td>
<td>Died in 100 days</td>
</tr>
<tr>
<td>No antibiotics ..........</td>
<td>106</td>
<td>105</td>
</tr>
<tr>
<td>Combined antibiotics*</td>
<td>132</td>
<td>131</td>
</tr>
<tr>
<td>Single antibiotic‡</td>
<td>369</td>
<td>342</td>
</tr>
<tr>
<td>Statistical evaluation by the 3 × 2 table method ....</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Streptomycin, kanamycin, or neomycin, combined with bacitracin.
† One case with sporeforming bacteria, 1 with yeast.
‡ Streptomycin, kanamycin, or neomycin alone.
§ One case with sporeforming bacteria, 1 with Klebsiella, 3 with Paracolobactrum, 3 with Alcaligenes fecalis, 3 with unidentified species.

* Streptomycin, kanamycin, or neomycin, combined with bacitracin.
† One case with sporeforming bacteria, 1 with yeast.
‡ Streptomycin, kanamycin, or neomycin alone.
§ One case with sporeforming bacteria, 1 with Klebsiella, 3 with Paracolobactrum, 3 with Alcaligenes fecalis, 3 with unidentified species.
antibiotic regimens or no antibiotic supply at all. Animals dying with uncontrolled secondary disease as well as those dying during treatments aimed at its suppression are included in this table. Both the combined and the single antibiotic regimen reduced the incidence of bacteremia below that in mice without antibiotic supply ($\chi^2 = 5.07, P < .05$; and $\chi^2 = 94.62, P < .01$, respectively), but the single antibiotic regimen was more effective than the combined one ($\chi^2 = 58.44, P < .01$). The bacteremia of mice receiving no antibiotics or a single antibiotic was usually caused by *Proteus sp.*, and less frequently by *E. coli*, enterococci, or streptococci. Administration of a single antibiotic (streptomycin, neomycin, or kanamycin) decreased slightly the incidence of bacteremia due to *Proteus* ($\chi^2 = 5.640, P < .05$), but increased the incidence of bacteremia with *E. coli* ($\chi^2 = 8.173, P < .01$), and also resulted in bacteremias caused by microorganisms absent from mice not receiving antibiotics, e.g., *Paracolobactrum, Alcaligenes fæcalis, Klebsiella*, and sporeforming bacteria. On the other hand, treatment with a combination of antibiotics eliminated *Proteus* and *E. coli* as causes of bacteremia. As compared with mice receiving no antibiotics and with those receiving the single antibiotic regimen, the incidence of bacteremia due to *Proteus* was significantly lower ($\chi^2 = 51.177, P < .01$; and $\chi^2 = 23.383, P < .01$, respectively). The combined antibiotic supply, however, frequently terminated in the appearance of antibiotic-resistant *E. cloacae* in the blood.

Mice supplied with antibiotics tolerated the immunosuppressive treatment aimed at control of the acute secondary disease better than mice without antibiotics. The single antibiotic regimen was superior to the combined one (table 2). (Although the 3 groups from this table include comparable proportions of mice subjected to each kind of immunosuppressive treatment, they are not strictly comparable because of differences in timing and dosage). In 2 experiments that were strictly comparable in that equal doses of cyclophosphamide were given on days 4–15 after irradiation and injection of allogeneic cells, only 3 of 21 mice supplied with streptomycin died within 3 weeks after irradiation, i.e., in the period of bone marrow aplasia and graft-versus-host reaction, whereas as many as 10 of 16 mice not supplied with antibiotic died in this period ($\chi^2 = 7.351, P < .01$). These 10 animals all died with bacteremia due to *Proteus* and *E. coli*, whereas the first 3 mice died with negative blood cultures ($P = .035$ by the Fisher's test).

**Discussion**

These observations can be summarized as follows. Oral administration of nonabsorbable antibiotics in high doses may eliminate completely the aerobic bacteria from the fecal flora of mouse radiation chimeras, but under conditions of incomplete isolation this “decontaminated” state disappears within 2 weeks because antibiotic-resistant bacteria occupy the digestive tract; the “selective” elimination of *Proteus* and *E. coli* from the fecal flora of the chimeras makes it possible to control the acute secondary disease (graft-versus-host reaction) by prolonged immunosuppressive treatment (administration of cyclophosphamide) without exposing the animal to the risk of bacteremia due to bone marrow aplasia.

The precautions for isolation employed in this

**Table 2.** The effect of supply of antibiotic on the mortality of leukemic mice subjected to irradiation and injection of allogeneic spleen cells, and then to different procedures aimed at suppression of the graft-versus-host reaction

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weeks after irradiation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td>No antibiotics (106 mice)</td>
<td>87.7*</td>
</tr>
<tr>
<td>Combined antibiotics† (132 mice)</td>
<td>62.9</td>
</tr>
<tr>
<td>Single antibiotic† (369 mice)</td>
<td>36.9</td>
</tr>
<tr>
<td>Statistical evaluation</td>
<td>$\chi^2 = 94.270$</td>
</tr>
<tr>
<td>by the 3 $\times$ 2 table method</td>
<td>$P &lt; .01$</td>
</tr>
</tbody>
</table>

* Cumulative mortality (%).
† Streptomycin, kanamycin, or neomycin, combined with bacitracin.
‡ Streptomycin, kanamycin, or neomycin alone.
study were evidently insufficient, since antibiotic-treated mice maintained under strict isolation in a horizontal laminar flow bench remain free of any aerobic fecal flora for as long as 40 days after sublethal irradiation. The antibiotic-resistant Enterobacteriaceae presumably originated from the experimental monkey rooms (located in the same building) where irradiated monkeys are treated systemically with antibiotics for longer periods of time.

Under these conditions of incomplete isolation, the “selective” elimination of Enterobacteriaceae from the fecal flora by streptomycin, kanamycin, or neomycin was advantageous in 2 respects. First, the “Enterobacteriaceae-free” chimeras were less likely to suffer from endogenous infection with *Proteus* or *E. coli*; second, they resisted exogenous infection more efficiently than did the animals whose aerobic fecal flora had been destroyed completely by the application of the combination of streptomycin, kanamycin, or neomycin, with bacitracin. The stability of the intestinal flora remaining after “selective” elimination of Enterobacteriaceae and its resistance toward exogenous contamination has been discussed in more detail elsewhere [7]. However, data reported here suffer from several drawbacks. First, most experiments concerned with suppression of the acute secondary disease by different means (see Materials and Methods) were not strictly paired in the chimeras that did or did not receive antibiotics, because at that time the main object was to treat leukemia and to have the animals survive the acute secondary disease, and the bacteriologic aspects of the supportive antibiotic treatment were not investigated in detail. Second, the relationship of radiation to treatment is different in the groups receiving combined versus the single antibiotic. Third, the role of anaerobic bacteria has not been clarified by the culturing techniques used. Fourth, the supply of streptomycin was usually alternated with a supply of kanamycin or neomycin in order to overcome resistance, a procedure that makes it impossible to compare the effect of streptomycin against anaerobes with that of the other 2 antibiotics. So far, we can only say that oral administration of a nonabsorbable antibiotic and barrier-nursing diminished the hazard of endogenous and exogenous infection with gram-negative bacteria during high-dose immunosuppressive treatment of radiation chimeras, and that similar treatment schedules employed in order to suppress the acute secondary disease were generally better tolerated by chimeras receiving a single, nonabsorbable antibiotic (streptomycin, kanamycin, or neomycin) than by those receiving a combination of nonabsorbable antibiotics (either of the 3 with bacitracin) or no antibiotics at all. Clarification of this point evidently needs a further, more detailed study. Similar data have recently been reported by Bruckner [10].

**Summary**

Irradiated leukemic mice, grafted with allogeneic spleen cells, received nonabsorbable antibiotics per os and received barrier-nursing. Their cages, bedding material, and food were autoclaved, and the animals were handled under aseptic conditions. These precautions were designed to improve the effectiveness of immunosuppression with cyclophosphamide, X-rays, or antidonor serum, which were used as treatment of the acute secondary disease in these chimeras. The combinations of streptomycin, kanamycin, or neomycin, with bacitracin eliminated complete aerobic flora that could be cultivated from the feces. Under conditions of incomplete isolation of the chimeras, as employed in this study, this “decontaminated” state usually terminated in uncontrollable infections with antibiotic-resistant bacteria from the environment. Treatment with a single antibiotic (streptomycin, kanamycin, or neomycin) eliminated primarily *Proteus* and *E. coli* from the fecal flora. This regimen reduced the incidence of bacteremia caused by *Proteus sp.* and *E. coli*, and permitted high-dose immunosuppressive treatment resulting in temporary bone marrow aplasia.

**References**


