Reports and Letters

Influence of the Concentration of Leukemic Inoculum on the Effectiveness of Treatment

Although the clincial treatment of acute leukemia with folic acid antagonists, first employed by Farber *et al.* (*1*), is often effective early in the course of treatment, it is generally considered that the ultimate failure of treatment is attributable to the emergence of resistant variants of the leukemic cells. This followed from the development by Burchenal *et al.* (*2*) and Law and Boyle (*3*) of mouse leukemias resistant to folic acid antagonists, such as amethopterin.

In this laboratory, leukemic mice were kept alive for an extended period of time with massive doses of amethopterin. The leukemic cells from such mice, however, showed on transplantation little, if any, evidence of resistance (*4*). It has also been observed that treatment of leukemic mice with an antagonist of folic acid late in the course of the disease was considerably less effective than treatment initiated early (*5*). The last-cited observations suggested that failure of treatment could result from an increased population of leukemic cells in the host (*4*). The current experiment (Fig. 1) demonstrates the importance of the number of leukemic cells in influencing the effectiveness of treatment.

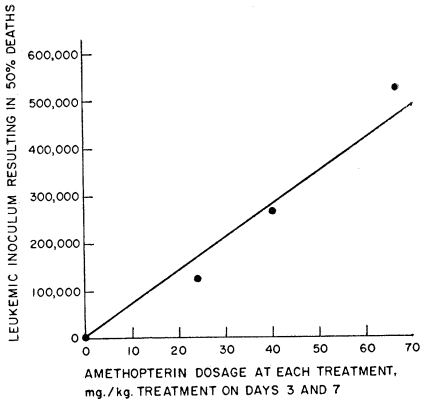

Fig. 1. Relationship between level of treatment with amethopterin and the size of the leukemic inoculum (cells per mouse) required to yield 50-percent mortality (EI_{50}). Early deaths from drug toxicity are excluded in computing the percentage mortality resulting from the leukemic inoculum. Without treatment, the EI_{50} was about 4000 cells.

The mice were inoculated intramuscularly with varying levels of leukemic (L1210) cells and were treated intraperitoneally with amethopterin on the third and again on the seventh day following inoculation with the leukemia. Three different dosages of amethopterin were employed, and, in addition, one set of leukemic mice was left untreated.

The mice were observed for mortality for 100 days following leukemic inoculation. Mice that survived 100 days were apparently "leukemia-free." Spleen implants from such mice did not result in leukemic growth. For each treatment level, and for the untreated group, the percentage of mice that died of leukemia increased with increasing inoculum. As the inoculum concentration was increased, it was apparently necessary to increase the dosage of drug to maintain the effect against the tumor.

Figure 1 shows the approximately linear relationship between the number of cells in the leukemic inoculum necessary to kill 50 percent of the mice (EI_{50}) and the dosage of amethopterin. From an EI_{50} of 4000 cells when no treatment was administered, there was an increase to more than 500,000 cells at the highest dosage of amethopterin employed, a relationship indicating that the level of treatment yielded better than 99-percent inactivation of the leukemic cells. The limitation to the continuation of this positive relationship is the toxicity of the drug to the host. At the highest dosage of the drug (67 mg/kg × 2), approximately 20 percent of the mice succumbed to drug toxicity. These animals were excluded from the calculations of the EI_{50}.

For a specific treatment, administered at a specified time after inoculation of the leukemia, this type of relationship describes the maximal potency of the drug. To improve treatment, it would be necessary to increase the effectiveness of the drug with respect to the leukemia without proportionately increasing the toxicity of the drug for the host. This may be accomplished, for example, by alteration of the schedule of treatment (*5, 6*) or by delayed administration of citrovorum factor (*6*).

The data indicate that the effectiveness of treatment is influenced by the number of leukemic cells in the host. Apparently, then, in addition to the possible emergence of resistant leukemic cells, and in addition to other factors, such as the possible diminution in the tolerance of the host to a particular treatment, progressive increase in the number of cells, despite treatment, may render a particular treatment progressively less effective. By the same token, the treatment of advanced leukemia may be hampered by the increased number of cells.

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References and Notes

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Natural Selection Associated with the ABO Blood Group

Geneticists have suspected and suggested that human blood groups should be subject to the processes of natural selection (*1*). Except for hemolytic disease of the newborn, no conclusive evidence that this was so had been brought forward until the article by Aird, Bentall, and Fraser Roberts appeared in 1953 (*2*). An extensive, well-controlled, clinical study involving population groups throughout England and Scotland demonstrated convincingly the existence of an association between carcinoma of the stomach, peptic ulceration, and the ABO blood group. This report is concerned with the preliminary results of a similar study conducted in the United States. Buchanan and Higley in 1921 (*3*) and Mayo and Fergeson in 1953 (*4*), in the only similar American studies, concluded that there was no relationship between blood groups and malignancy and none between blood groups and any disease for which sufficient data were available to justify a conclusion.

The case material utilized in the study reported here was provided by