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where $k$ is the experimentally determined fraction of plasma radioactivity excreted per day (*15*).
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## Immunization against Rauscher Mouse Leukemia with Tissue Culture Material

Abstract. *Long-term monolayer cultures of mouse thymus and spleen cells, chronically infected with Rauscher virus, showed only a very attenuated leukemia-producing capacity. Material from these cultures prepared from normal BALB/c mouse tissue was used to immunize isologous mice against subsequent challenge with fully active Rauscher virus. The centrifuged supernatant of the culture had some immunizing properties. However, the most efficient protection was obtained when cell suspensions from these cultures were used in the form of a series of three intraperitoneal and intramuscular injections and one booster injection.*

Immunological reactions of the host in leukemias of the Friend and Rauscher types have been reported. Initially Friend (*1*) demonstrated that previous inoculations of susceptible mice with formalin-treated extracts from leukemic spleens could protect against challenge with the active virus. Fink and Rauscher (*2*) obtained similar results with the Rauscher virus. Specific new antigenic components in the Friend leukemia have been demonstrated by cytotoxic tests in vitro (*3*) and with the aid of immunofluorescence techniques (*4*).

The aim of the experiments reported here was to explore the possibility of protecting mice against leukemia by immunization with long-term infected cultures of normal lymphoid mouse cells; the cultures were latently infected with the Rauscher virus and showed an attenuated leukemia-producing capacity.

The original strain of Rauscher virus was given to us by Dr. Rauscher. The currently used virus stock was prepared from a centrifuged homogenate (10 percent in 0.153*M* potassium citrate) of spleens from diseased animals. Its titer, as determined by intraperitoneal inoculations of 1-month-old BALB/c mice, was of the order of $10^6$ $LD_{50}$ (lethal dose, 50 percent effective) per milliliter. Mice of the BALB/c strain, aged 1.5 to 2 months, were used uniformly throughout these experiments. The development of the Rauscher disease in the virus-inoculated animals was checked routinely by spleen palpation and hematological examination. Satisfactory agreement was established between the estimation of splenomegaly in living animals and the spleen weights as verified in killed mice. In general, palpation, scored from 0 to plus 4, corresponded to spleen weights of less than 0.3 g, 0.3 to 0.6 g, 0.6 to 1 g, 1 to 2 g, and more than 2 g.

The V5 cell line developed in vitro by Wright and Lasfargues (*5*) from mixed BALB/c spleen and thymus cultures was infected with the Rauscher virus and reproduced the virus actively for more than 80 passages, apparently without harm to the reticulum-type cells multiplying rapidly in these cultures. In our laboratory the V5 line was cultivated in NCTC 109 synthetic medium supplemented with 20 percent calf serum.

In November 1964, the supernatant of the V5 cultures, diluted 10 times or undiluted, was inoculated intraperitoneally at a dosage of 0.1 ml into 16 BALB/c mice, aged 1 to 2 months. No leukemias were observed in any of the inoculated animals. At the same time, cells from the cultures were inoculated intraperitoneally into five BALB/c mice of the same age, at dosages of 1 million cells per mouse. Again no symptoms of the Rauscher disease were noted in any of these animals.

Consequently, experiments were designed to check whether mice that received prior inoculation of the culture supernatant and then remained symptom free would resist challenge with the fully active Rauscher virus.

In two independent series, 0.1 ml quantities of the V5 culture supernatant were inoculated into 1-month-old BALB/c mice. About 23 or 27 days later the mice, all of which appeared to be healthy, were inoculated with the infectious stock virus in parallel with control animals (Table 1).

As can be seen from the table, half of the animals that had been inoculated with tissue culture supernatant were protected against the challenge with relatively high doses of virulent virus.

In a parallel series, 13 mice inoculated with only the culture supernatant remained apparently healthy during the 150-day observation period.

Since these results were indicative

Table 1. Results of inoculation (challenge) of Rauscher virus into control mice and mice that received prior treatment with V5 supernatant. The incidence of splenomegaly in each group is recorded out of the total number of mice in each group.

| Days after challenge | | | | | | | Survival 150 days after challenge |
|---|---|---|---|---|---|---|---|
| 5 | 9 | 13 | 19 | 22 | 26 | 32 | |
| *Control mice* | | | | | | | |
| 0/10 | 5/10 | 9/10 | 10/10 | 10/10 | 9/9 | 9/9 | 0/10 |
| *Mice with prior inoculation with culture supernatant* | | | | | | | |
| 0/14 | 3/14 | 6/14 | 7/14 | 7/14 | 7/14 | 7/14 | 7/14 |

Table 2. Results of immunization of BALB/c mice with supernatant and cells from V5 cultures. The material indicated was inoculated on days 1, 4, and 7 intramuscularly and intraperitoneally, and on day 22 intraperitoneally. Groups III, IV, and V were challenged with virulent Rauscher virus on day 28. Groups I and II were not challenged. CS, culture supernatant.

| Mice | | Material inoculated | Leukemic animals*/ total |
|---|---|---|---|
| Group | No. | | |
| I | 9 | CS | 3/9 |
| II | 10 | CS and cells | 0/10 |
| III | 10 | Stock medium | 8/10 |
| IV | 19 | CS | 9/19 |
| V | 19 | CS and cells | 0/19 |

* Observations at the 55th day after challenge based on spleen palpation and hematological examinations.

Table 3. Splenomegaly in randomly chosen mice challenged with Rauscher virus. A similar group of mice (group V) immunized with V5 cultures showed no evidence of disease under identical conditions. Each entry represents one animal, the reactions being graded from − to ++++.

| Days after challenge with active virus | Controls from group III, inoculated with plain medium | | | | |
|---|---|---|---|---|---|
| 7 | − | − | − | − | − |
| 14 | + | + | − | + | + |
| 18 | +++ | + | + | ++ | ++ |
| 22 | ++++ | ++ | ++ | +++ | ++ |
| 26 | ++++ | +++ | ++ | +++ | +++ |
| 30 | died | ++++ | ++++ | ++++ | ++++ |
| 33 | | ++++ | ++++ | ++++ | ++++ |
| 37 | | +++ | ++++ | ++++ | ++++ |

of a protective effect of the prior inoculation with tissue culture material against the subsequent challenge with the infective virus, further experiments were undertaken to confirm these data and especially to evaluate the immunizing effect of the supernatant as compared with the cell constituent of the V5 cultures.

A uniform group of BALB/c mice aged 1.5 to 2 months was divided into five subgroups and treated as indicated in Table 2.

The supernatant used for immunization was taken from proliferating cultures 3 days after feeding with new medium. The material was centrifuged for 30 minutes at 3000 rev/min.

The cells were collected from monolayer cultures, centrifuged at 1500 rev/min for 15 minutes, and resuspended in their medium at a density on the order of $3 \times 10^6$ cells per ml. The cell suspension was frozen at 70°C and thawed three times before use.

The scheme of the immunizing injections, with the supernatant culture fluid, the cell suspension, or, as a control, with the stock culture medium, was as follows: each animal received 0.5 ml intraperitoneally and 0.25 ml intramuscularly at the 1st, 4th, and 7th days. This series was followed by one intraperitoneal booster injection on the 22nd day. All animals were challenged with 0.1 ml stock virus intraperitoneally on the 28th day (Table 2).

The progress of the Rauscher disease in the inoculated animals as checked by spleen palpation was confirmed by hematological examinations. The splenomegalic animals in groups I, III, and IV had, 37 days after challenge, average leukocyte counts of 64,000, 60,000, and 75,000 cells per cubic millimeter, respectively, as compared with an average value of 8600 ± 4000 in the mice that showed no splenomegalic symptoms. In addition, all the diseased mice had large numbers of erythroblasts in their blood.

The course of the disease in randomly chosen individual mice in the immunized group V and in control group III is shown in Table 3. The culture supernatant alone was capable of producing leukemias in mice to some extent. (Table 2). Nevertheless, it conferred a certain amount of protection against challenge with the fully active virus (group IV). However, the most significant degree of protection was noted in the group of animals that had been immunized with the *cell fraction* from the latently infected cultures (group V).

In agreement with the observations made by Wright (*6*), the leukemia-producing capacity of long-term spleen-thymus cultures infected with Rauscher virus became attenuated considerably after 1 year or more of continuous passages in vitro. However, this capacity did not disappear entirely, since we found that the supernatant of these cultures produced leukemia if inoculated in massive, repeated doses by the intraperitoneal and intramuscular routes into young adult BALB/c mice.

The assumption that previous inoculation with the attenuated tissue culture material, when not producing any recognizable disease, can protect the treated animals against subsequent inoculation with active Rauscher virus was fully confirmed by our data.

In our first experiments, intraperitoneal injections of the supernatant from permanently infected cultures protected a significant proportion of young mice against challenge with the virulent virus and prevented the occurrence of Rauscher disease during an observation period of 150 days. Under our experimental conditions, this most probably represents definite protection.

However, what appears to us of particular interest is the effect of immunization with the supernatant as compared to immunization with the cell material from the V5 cultures. The repeated inoculations with frozen and thawed cells from these cultures were by far more efficient, giving 100-percent protection in the treated mice.

Although the culture supernatant, when repeatedly injected, showed some residual potency in inducing Rauscher disease, curiously enough, no leukemias were produced when cells in massive doses were present in the inoculum. These data, when considered as a whole, suggest that the essential immunizing capacity of the isologous spleen-thymus cultures, chronically infected with Rauscher virus, resides in the cells.

There can be different explanations for this. The cells may have been supplying much more of the viral immunizing material in the form of "attenuated" or incomplete virus than the supernatant and, in the cells, the noninfectious but immunizing factor may have been dominant. But it might also be assumed that in the induction of immunity against the Rauscher disease the latently infected spleen-thymus cells were themselves supplying a new antigen essential and efficient in the immunization process.

Regardless of the underlying explanations, our data indicate the possibility of using long-term cultures of lymphoid tissue in vitro containing an attenuated leukemia-producing factor and especially the cellular component of these cultures to induce efficient immunity and to prevent the evolution of the malignant disease.

GEORGES BARSKI
JUNG KOO YOUN
*Institut Gustave Roussy, Villejuif (Seine), France*, and *Sloan-Kettering Institute for Cancer Research, New York*

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