ONCOLOGY

THE ANTIGENETIC PROPERTIES OF "HUMAN LEUKEMIC FACTOR"
CULTIVATED ON THE CHORIOALLANTOIC MEMBRANE
OF THE CHICK EMBRYO

V. M. Bergol'ts and L. V. Shershul'skala

From the Virologic Laboratory of the P.A. Gerssen State Oncological Institute
(Director – Prof. A. N. Novikov; Scientific Adviser – Corr. Member Acad. Med. Sci. USSR
A. I. Savitskii), Moscow

(Received December 1, 1957. Presented by Active Member Acad. Med. Sci. USSR L.A. Zil'ber)

In the study of the properties and nature of human "leukemic factor" [1] the possibility of its cultivation
on the membranes of the developing chick embryo is particularly important.

In order to prove that this agent may be actively cultivated on the membranes of the chick embryo three
main methods are employed: testing the specific biological activity of the culture material in animals, study of
the culture material with the electron microscope and determination of its specific antigenic properties in im-
munological reactions. In this report we give the results of a study of the specific antigenic properties of the
leukemic factor.*

As we know from the work of L. A. Zil'ber, V. A. Parnes [3] and others, a specific leukemic antigen is pre-
sent in the bodies of leukemia patients.

The main purpose of our work was to find out: does the allantoic fluid of an embryo in which blood from
leukemia patients has been inoculated contain a factor analogous to the specific leukemic antigen contained in
the blood of patients with leukemia?

We found no evidence in the literature of the possibility of cultivation of material obtained from leukemia
patients in the developing chick embryo.

EXPERIMENTAL METHOD

Blood from patients with acute leukemia was cultivated on the chorioallantoic membrane of seven-day
chick embryos. Inoculations were made once every five days using cortisone.

For detection of the leukemic factor and determination of its specific antigenic properties we used one of
the most sensitive immunological reactions – L. A. Zil'ber’s active anaphylaxis and desensitization reaction. The
method of performing this reaction did not differ in principle from that used by L. A. Zil'ber and his co-workers
[2, 3 and others] for demonstrating the specific antigens of malignant tumors (see Tables 1 and 2).

As antigens we used blood of leukemia patients, blood of healthy persons, allantoic fluid inoculated with

*The results of an investigation into the leukemia-producing activity of the cultivated agent together with a de-
scription of the method of its cultivation on the membranes of the chick embryo will appear in later reports.
blood* or marrow filtrate of leukemia patients; or allantoic fluid inoculated with blood of healthy persons.**

Altogether 90 guinea pigs (weighing 250-300 g) were sensitized. The tables show the results of experiments on 70 guinea pigs; the remaining animals died or were removed from the experiment for various reasons. The protein content (by Kjeldahl's method) of the allantoic fluid inoculated with leukemic blood was 2.88 mg/ml; that of allantoic fluid inoculated with normal blood was 2.66 mg/ml.

**EXPERIMENTAL RESULTS**

The object of the first series of experiments was to find out if there were any specific antigenic differences between "leukemic" and "normal" allantoic fluid.

Guinea pigs which had been sensitized to "leukemic" allantoic fluid and desensitized to "normal" allantoic fluid were tested for the presence of specific sensitization by giving them an assailing injection of "leukemic" allantoic fluid. Out of seven guinea pigs sensitized with a dose of 1.5 ml, a clear reaction was observed in five; in one animal the reaction was indefinite and in one it was not observed at all (Table 1, Experiment 8). Of seven guinea pigs sensitized with a dose of 1 ml, two showed a definite reaction, three an indefinite reaction and two no reaction (Table 1, Experiment 7). The best results were obtained by sensitization of guinea pigs with 2 ml of allantoic fluid inoculated with marrow filtrate from a leukemia patient.

After desensitization to allantoic fluid inoculated with filtrate of normal marrow tissue, an assailling injection of the antigen used for sensitization caused a clear anaphylactic reaction (Table 1, Experiment 10). These experiments revealed the decisive importance of the sensitizing dose and the time of sensitization: with large doses and a longer period of sensitization the anaphylactic reaction was more pronounced. This work also showed that it was better to use fresh than preserved antigen for the assailling injection.

The results of these experiments provide evidence of the presence of a specific factor in "leukemic" allantoic fluid which is absent from "normal" allantoic fluid.

It was necessary to prove that this is the "leukemic" factor and not some chance contaminating antigen. For this purpose the following series of experiments was performed.

Guinea pigs were sensitized with fresh whole blood from patients with acute leukemia. Desensitization was carried out with healthy human blood or with allantoic fluid in which healthy human blood had been inoculated.

An assailling injection of allantoic fluid inoculated with blood from a patient with leukemia caused a definite anaphylactic reaction in all 16 guinea pigs, which was not observed in control animals (Table 2, Experiments 2, 4, 5).

In this experiment attention is drawn to the absence of reaction in guinea pigs sensitized to leukemic blood on the injection of allantoic fluid inoculated with healthy human blood.

Thus, from the findings given it may be concluded that "leukemic" allantoic fluid contains a specific leukemic factor which is absent from "normal" allantoic fluid. As an additional control we made a special experiment in which guinea pigs sensitized with healthy human blood showed no anaphylactic reaction in response to injection of "normal" allantoic fluid nor to an assailling injection of allantoic fluid inoculated with blood or medullary filtrate from a patient with leukemia.

The results of this experiment, as of Experiments 5 (Table 2) and 9 (Table 3) indicate that the components of normal blood cannot be cultivated on the membranes of the developing chick embryo.

Having discovered that in animals sensitized with blood of leukemia patients no reaction is observed to "normal" allantoic fluid, we performed the converse experiment. Guinea pigs were sensitized with "leukemic" allantoic fluid, and received as an assailling injection blood from patients with leukemia or healthy human blood (Table 3).

This experiment gave clear results: while reaction to injection of healthy human blood into the guinea pigs was completely absent, a definite anaphylactic reaction was observed to injection of blood of the patient with leukemia.

---

*In the future this will be referred to briefly as "leukemic" allantoic fluid.

**In the future — "normal" allantoic fluid.