EXPERIMENTAL IMMUNIZATION OF HORSES WITH HELA CELLS

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The difficulties in obtaining specific anti-cancer sera [1-4, 6] necessary for carrying out immunological investigations of this disease, are well known. Attempts to use cancer cell cultures for immunization are of obvious interest in this connection. Particular attention of the investigators [8, 10-15] was directed to the HeLa strain cells, obtained from the squamous carcinoma of human cervix [12]. Antibodies reacting with HeLa cells antigens, have been found in the blood of cancer patients.

Antibodies to the cells of this strain have been obtained in rabbits [11, 13-15], guinea pigs [10] and rats [14]. The titer of rabbit antibodies in antisera was sufficiently high (in complement fixation reaction-CFR-1:1280). However, the question of obtaining antibodies in the course of animal immunization as well as the possibility of using large animals for immunization, remained almost untouched.

In this connection, we carried out experimental immunization with HeLa cells, studying the dynamics of antibody formation.

MATERIALS AND METHODS

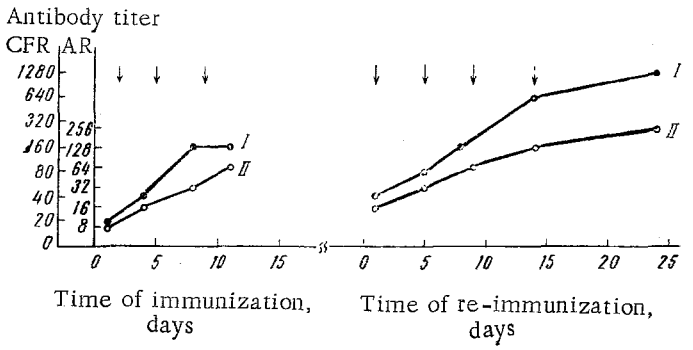
HeLa cells were grown in large glass containers, 1 to 5 liters in volume. Medium No. 199 with 10% native beef serum was used as the nutrient medium. The containers (Roux flasks and Povitskoi containers) with the inoculated cultures were placed for 7 days in a walk-in incubator at 36°. The grown cells were removed from glass with versene, and cells sedimented after centrifugation were collected and saved at -10°. A total of 8 transfers of the culture have been made during 2 months and almost $2 \cdot 10^{10}$ cells (over 20 g) have been collected.

A 7-year old stallion (No. 16) weighing 505 kg has been used for immunization.* Immunization and re-immunization were carried out using somewhat different procedures however, each time, blood samples were taken prior to, during and subsequent to immunization.

The immunization consisted of three parenteral injections of antigen with 3-4 day intervals. A 5% suspension of frozen HeLa cells in Hanks' solution, served an antigen. The dose of injected antigen was increased with each injection: 1st injection - 17 ml; 2nd - 35 ml; 3rd - 40 ml. Beginning with the 2nd injection we carried out desensitization according to Bezredka, using 1/7 of the average dose. Regardless of these precautions, the horse responded with considerable reaction to the last 2 injections; as a result of this, additional injections have not been carried out.

After an 8 month interval and additional cultivation of HeLa cells (about $5 \cdot 10^8$) the horse was re-immunized. The procedure consisted of 4 subcutaneous injections of antigen 4-5 days apart in 4-5 points on the back. A water-saline extract of HeLa cells was used as an antigen, and the amount injected was quantitated in mgs of protein; the amount of protein was determined from the amount of nitrogen, according to Conway's technique. For each

* The horse has been immunized in the N. F. Gamalei Institute of Microbiology and Epidemiology.



The rate of appearance of antibody to HeLa cells in the blood of horse No. 16. I) Titer of complement-fixing antibodies (CFR); II) titer of agglutinating antibodies (AR) (arrow indicates time of injection of antigen).

injection we used 10-15 ml of antigen, allowing 0.4 mg protein per 1 kg body weight of the horse. The blood for analysis was obtained prior to both cycles of immunization as well as during the immunization: before the 2nd, 3rd, and 4th injections of antigen, and also on the 4th or the 10th day following the immunization cycle. The obtained sera were tested using the CF test at 37° [5] and a modified agglutination test [3, 6, 7]. The experimental results were evaluated according to the titers, i.e., the greatest dilution of serum giving a positive reaction with HeLa cells antigen (with 1 or 2 plus).

EXPERIMENTAL RESULTS

The summary of experimental data of titers of the horse antisera before immunization and prior to the anamnestic response, in the course of their development and at the end of the process, are presented in the figure.

During immunization the titer of complement fixing antibodies to the HeLa cells antigen increased slowly and did not reach high values (did not exceed 1:160). It decreased significantly before re-immunization (to 1:40), but showed a rapid and significant increase (up to 1:1280), as a result of 4 injections of antigen. Similar fluctuations in antibody titers to HeLa cells were observed in agglutination reactions, in which the titer of agglutinating antibodies did not exceed 1:256.

Our experiments, in which a horse has been used for the first time for immunization with cultures of HeLa cells demonstrated that antisera containing sufficiently high concentration of complement-fixing antibodies to HeLa cells could be obtained after re-immunization. It should be pointed out that in studies by other investigators [4, 7] immunizing horses with extracts of malignant human tissues, the titers of complement fixing antibodies to homologous antigens usually reached a moderate level (not over 1:320 to 1:640). The specificity of produced antibodies is of considerable importance, since usually anti-cancer sera are polyvalent in nature. Additional investigations were carried out to clarify this question; the results of these studies will be reported in subsequent communications.

SUMMARY

A horse was immunized with the cells of a culture of human carcinoma of the cervix tissue (HeLa strain).

The rise in the antibody titer against the HeLa strain was studied during the process of immunization and re-immunization in experiments on agglutination and complement-fixation. The most rapid rise in the antibody titer was observed following re-immunization which attained the greatest value after 4 injections of the antigen.

LITERATURE CITED

1. N. N. Zhukov-Verezhnikov, I. N. Maiskii, and V. S. Gostev, in: Development of Malignant State; Moscow (1959), p. 147.
2. L. A. Zil'ber and G. I. Abelev. Virology and Immunology of Cancer, Moscow (1962), p. 224.
3. M. M. Kapichnikov, Byull. Éksper. Biol. (1956), No. 3, p. 63.
4. I. N. Maiskii, P. P. Filatov, and G. V. Suvorova, Ibid. (1961), No. 4, p. 92.

5. I. I. Podoplelov, Ibid, (1959), No. 1, p. 52.
6. I. I. Podeplelov, Ibid, No. 6 p. 91.
7. A. K. Saakov. In: Immunological Methods for Studying Malignant Development, Moscow (1959), p. 203.
8. G. P. Tribulev and I. I. Podoplelov. Byull.Éksper. Biol. (1964), No. 6, p. 73.
9. W. S. Blakemore and J. M. McKenna, Surgery (1962), v. 52, p. 213.
10. K. G. Brand, Nature (1962), v. 194, p. 752.
11. L. L. Coriell, M. G. Tall, and H. Gaskill, Science (1958), v. 128, p. 198.
12. G. O. Gey, W. D. Coffman, and M. T. Kubjcek, Cancer Res. 952, v. 12, p. 264.
13. G. Goldstein, Q. N. Myrvik, J. Immunol. (1958), v. 80, p. 100.
14. R. M. McAllister, P. W. Grunmeier, and L. L. Coriell, et al., J. nat. Cancer Inst. (1958), v. 21, p. 541.
15. J. M. McKenna, R. P. Sanderson, and W. S. Blakemore, Science (1962), v. 135, p. 370.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. ***Some or all of this periodical literature may well be available in English translation.*** **A complete list of the cover-to-cover English translations appears at the back of this issue.**
