In Vitro Demonstration of an Antigen in Red Blood Cells of C3H Mice*

Lucie Adelsberger and H. M. Zimmerman

(Laboratory Division, Montefiore Hospital, New York, N.Y.)

In previous experiments with the spontaneous C3H mouse mammary tumor it was observed that antisera produced in rabbits with filtrates from this tumor inhibited the hemolytic effect of 10 per cent saline suspensions of mammary tumors on mouse red blood cells (2). This inhibition was achieved only by sensitization of the mouse red blood cells, which in the nonsensitized state were hemolyzed by the tumor suspension. It was not achieved by mixing the antitumor serum with the tumor suspensions. The inhibition produced by the antimammary tumor serum was, on the average, 8 times greater than that produced by normal rabbit serum. These findings suggested further studies with antisera produced in rabbits with other mouse tumor filtrates, to determine whether this inhibition of hemolysis by suspensions from mammary tumors is specific for the antimammary tumor serum. Accordingly, experiments were made with antitumor rabbit sera prepared with two different brain tumors induced in mice with methylcholanthrene (6): (a) an undifferentiated malignant glioma grown in the C3H mouse strain (which is susceptible to spontaneous mammary tumor) and (b) an ependymoma grown in the C57BL strain (which is resistant to mammary tumor).

It has also been found that there are striking differences in the hemagglutinating and hemolytic behavior between the red blood cells of the C57BL mice, which are mammary tumor-resistant, and those of the C3H mice, which are susceptible (1, 2). And, most significantly, there is a difference between the red blood cells from tumor-bearing and those from nontumor-bearing animals of comparable ages (over 1 year) of the C3H strain. Thus, the questions arise: (a) Does this difference in the hemagglutinating and/or hemolytic behavior represent a difference in the antigenic constitution of these red blood cells; and (b) is there a relationship between the antigenic constitution of these red blood cells and the tumor? To investigate these points, rabbit antisera were prepared with red blood cells from nontumorous C57 mice, from nontumorous C3H mice, and from C3H mice bearing spontaneous mammary tumors. These antisera were tested in the same manner as the antitumor rabbit sera for inhibition of the hemolytic effect of suspensions of mammary tumors on mouse red blood cells.

MATERIALS AND METHODS

C3H and C57BL mice from our own breeding colony were employed for tumor growth and for bleeding as previously described (1). The tumors used for immunization of rabbits were:

1. C3H(19), an undifferentiated, malignant glioma originally induced in a C3H mouse with methylcholanthrene and carried on by subcutaneous transplantation in the C3H strain. This tumor grew rapidly and killed the mice in an average of 30 days after transplantation. The tumor was employed in the 63rd–65th passage.

2. C57(26), an ependymoma originally induced with methylcholanthrene in a C57BL mouse and carried on by subcutaneous transplantation in the C57BL strain. This tumor was used in the 55th passage. It had a much slower rate of growth than the C3H(19) tumor but finally killed the mice in an average of 100 days.

3. Spontaneous mammary tumors from C3H mice were used for the control sera.

Silk filtrates of all tumors were prepared as previously described (6).

The erythrocytes used for immunization were obtained from (a) nontumorous C3H mice, (b) nontumorous C57BL mice, and (c) C3H mice with spontaneous mammary tumors in various stages of growth. Animals of both sexes were employed and, with few exceptions, at 13–20 months of age. Ten per cent red blood cell suspensions were used. The red blood cells were always kept on ice and employed within 60–90 minutes after bleeding.

The rabbits were immunized with either the tumor filtrates or the red blood cell suspensions in exactly the same manner as to dose and interval of injection: One intravenous injection of
1 cc. of tumor or red blood cell material was followed by four intraperitoneal injections of 5 cc. However, with the ependymoma C57(26) filtrates, one intravenous and only three intraperitoneal injections were given because of lack of material. Blood from the rabbits was drawn from the heart 3-4 weeks after the last injection. The complement of the sera was inactivated at 56° C. for 30 minutes, and the sera were then stored at −20° C. for various lengths of time. The control sera from normal rabbits were treated in the same manner.

Inhibition of the hemolytic effect of suspensions from mammary tumors on mouse red blood cells sensitized with the rabbit antisera was determined as previously reported (2). The red blood cells were sensitized in the following manner:

RESULTS

The results are summarized in Chart 1. It is evident that the inhibition of the hemolytic effect of suspensions of mammary tumors on mouse red blood cells was not specific for the antimammary tumor serum. Inhibition was approximately the same when the mouse red blood cells were sensitized with antitumor rabbit sera prepared with glioma CSH(19) or ependymoma C57(26) filtrates, or with antitumor rabbit sera prepared from CSH mammary tumors. The titer of inhibition was in the range of 32-64, with some minor deviations to either side, and corresponded to the titer observed previously with antimammary tumor rabbit sera. If very slight differences are considered, the anti-CSH(19) serum had a somewhat better inhibitory effect than the antimammary tumor serum; anti-

CHART 1.—Titer of inhibition of hemolysis of mouse red blood cells by mammary tumor suspensions after sensitization

Two-tenths cc. of blood cell suspension was added to 0.2 cc. of twofold serial saline dilutions of antitumor rabbit serum or anti-RBC serum and to corresponding dilutions of normal rabbit serum, respectively. The mixtures were incubated for 60 minutes at 37° C. and then for 90 minutes at 4° C. They were then centrifuged for 4 minutes at 2,000 r.p.m., and the supernatant sera were discarded. The red blood cells were taken up in 3 cc. of cold sterile saline, centrifuged again for 4 minutes at 2,000 r.p.m., and freed from the saline. Afterwards, the hemolytic effect of the tumor suspensions was tested on the sensitised mouse red blood cells. Ten per cent tumor suspensions were freshly prepared with cold saline at 15° C., and the supernatant material employed in the tests was obtained by centrifugation at a radius of 4 inches and at 3,250 r.p.m. for 6 minutes at room temperature. In control tests, saline instead of the red blood cells with rabbit antisera. Each symbol indicates one test read after 3-5 hours.
Sensitization of the mouse red blood cells with anti-red blood cell sera inhibited the hemolytic potency of tumor suspensions differently according to the origin of the red blood cells used for immunization. After sensitization with anti-C3H red blood cell sera, the titer of inhibition was 32 in seven instances, 64 in thirteen instances and 128 in two instances. After sensitization with anti-C57 red blood cell serum, the titer of inhibition was 16 in six instances, 32 in ten instances and 64 in six instances. After sensitization with anti-C3H red blood cell serum (produced with red blood cells from C3H mice bearing spontaneous tumors and designated Tum C3H), the titer of inhibition was 32 in five instances, 64 in thirteen instances, and 128 in five instances. In control tests, after sensitization of the mouse red blood cells with normal rabbit sera, the titer of inhibition was 4 in nineteen instances, 8 in fourteen instances, and 16 in one instance. Thus, it is evident that the titer of inhibition of hemolysis produced by anti-C3H red blood cell sera was about the same as that of the antitumor sera tested. Sensitization with anti-C57 red blood cell sera, however, produced a definitely lower titer of inhibition. Sensitization with the anti-Tum C3H red blood cell sera gave the strongest inhibition observed with any antisera employed in this series, obviously even stronger inhibition than with the antitumor sera.

In each test, red blood cells from nontumorous C3H and C57 mice and from C3H mice with spontaneous mammary tumors were used for sensitization. There was no remarkable difference between the red blood cells at readings after 8-5 hours. At readings after 15-18 hours, sensitized C57 and Tum C3H red blood cells reacted about the same, but sensitized C3H red blood cells frequently showed a twofold lower titer.

In the control series, wherein saline instead of tumor suspension had been added, hemolysis was not observed after 24 hours. After 48 hours hemolysis of C3H red blood cells occurred with higher serum dilutions. Occasionally it also occurred with Tum C3H red blood cells, but not with C57 red blood cells.

DISCUSSION

From the data presented it is evident that the inhibition of the hemolytic effect of suspensions from mammary tumors on mouse red blood cells is not specific for the antimammary tumor serum alone. Inhibition has been observed to the same degree with antitumor sera prepared with filtrates from tumors of different origin and also with anti-tumor sera prepared with filtrates from a tumor of a different mouse strain.

With anti-erythrocyte sera the findings are even more striking. The anti-C3H red blood cell sera caused inhibition of the hemolytic effect of tumor suspensions on mouse red blood cells in the same manner and to the same degree as antitumor sera. By contrast, the anti-C57 red blood cell sera showed definitely less inhibition than the anti-C3H red blood cell sera and all the antitumor sera tested. This is significant, because four rabbits were employed in the preparation of each of the antisera and also because, in the paired tests performed with C3H and C57BL red blood cell antisera, the titer was invariably lower with the antisera of the latter mouse strain. These findings indicate that the C3H red blood cells have an antigen in common with or related to the tumor which the C57 red blood cells do not have. The presence of an antigen in mouse red blood cells of tumor-susceptible animals has been suggested by Gorer (5). He found that the red blood cells from the A strain of mice have at least one antigen in common with normal and neoplastic tissues of this strain. Recently, while the present work was in progress, Barrett contributed more data on this problem (3, 4). In mice with differing genetic constitution, he induced resistance to tumor growth by prior injection of red blood cells derived from the strain in which the tumor had originated. Thus, the results of the present study, although obtained with antisera produced with crude antigens, confirm the findings of Gorer and Barrett from another angle. Attention should be drawn, however, to the fact that in the current experiments the presence of an antigen related to the tumor was demonstrated in vitro, without tumor transplantation into the animals. This approach may offer a broader avenue for the investigation of tumor antigens in general.

Finally, the question arises whether the antigen found in the C3H red blood cells but not in the C57 red blood cells is related to the hemolytic factor observed with the C3H but not with the C57 erythrocytes. This question needs further investigation. One fact must be borne in mind: namely, that there is a relatively high titer of inhibition of hemolysis by antisera prepared with erythrocytes from tumor-bearing C3H mice, and only a limited hemolysis of the erythrocytes of these animals with dilutions of normal rabbit serum. Therefore, it seems unlikely that there is a relationship between the antigen and the hemolytic factor. Theoretically, however, it may be assumed that red blood cells from tumor-bearing mice acquire an antibody against the tumor in addition to the hemolytic factor which they possess. This anti-
body can act like the one which inhibits hemolysis of the red blood cells by tumor suspensions. Thus, the difference between the red blood cells from tumorous and nontumorous C3H mice would not depend so much upon a difference in the antigenic constitution as on the antibody content.

SUMMARY

1. Antitumor rabbit sera prepared with filtrates from glioma C3H(19), from ependymoma C57(26), and from spontaneous mammary tumors inhibit the hemolytic effect on erythrocytes by mammary tumor suspensions in approximately the same titer.

2. Anti-C3H red blood cell sera produce the same titer of inhibition as these antitumor sera.

3. Anti-C57 red blood cell sera have a slightly lower inhibitory effect than anti-C3H red blood cell sera and antitumor sera.

4. Antisera prepared with red blood cells from C3H mice with spontaneous mammary tumors produce the strongest inhibition.

5. The implications of these findings obtained by in vitro experiments are discussed.

REFERENCES


2. ———. Differences in the Hemolytic Behavior of Red Blood Cells of a Tumor-susceptible (C3H) and a Tumor-resistant (C57) Mouse Strain. Ibid., pp. 658-62.


In Vitro Demonstration of an Antigen in Red Blood Cells of C3H Mice

Lucie Adelsberger and H. M. Zimmerman


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/13/7_Part_1/521

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.