IMMUNODIFFUSION ANALYSIS OF MOUSE LEUKEMIA ANTIGENS

O. M. Lezhneva

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A precipitating serum against type-specific antigen of Rauscher and Friend leukemias was obtained by immunizing rabbits with intact Rauscher virus. The immunologic specificity of the precipitating antigen was studied by immunoautoradiography. A precipitating test system for the type-specific antigen of Rauscher and Friend leukemias was obtained.

The specific antigens of the mouse leukemias have been studied for more than 10 years, yet their nature has not yet been explained. It has proved impossible by combined immunologic and electron-microscopic investigations using the sera of mice immunized with leukemic cells to differentiate the specific antigens of the cell membrane from antigens of the virus particle [3, 12], for these sera contain both cytotoxic and virus-neutralizing antibodies [13]. These antigens can be differentiated only by means of monospecific antisera.

Monospecific antibodies, especially against individual antigens of the virus particle, are also of great interest as a means of studying vertical transmission of viruses [11] and the serotypes of mouse leukemic viruses [8].

The object of this investigation was to obtain a precipitating test system against the type-specific antigen of Rauscher's leukemia. To make the gel-diffusion method more sensitive, indirect immunoautoradiography was used [7].

EXPERIMENTAL METHOD

Rauscher Leukemia Virus (RLV). Two types of RLV were used: 1) a commercial preparation of RLV with a concentration of virus particles of 10^{-10} to 10^{-11}/ml (Virtgoreagents, Electro-Nucleonic Laboratories Inc.); 2) RLV isolated by the writer from the plasma of affected BALB/c mice by differential centrifugation in a sucrose density gradient [3].

Antisera. Antiserum 899 was prepared by immunizing a rabbit with intact RLV isolated from 17 ml of plasma. The concentrated virus (0.1 ml) was emulsified in 0.4 ml of Freund's complete adjuvant by Goudie's method [4, 10]. The material was injected in a volume of 0.1 ml directly into the lymph glands and in the same dose into the tissue surrounding them. Reimmunization was carried out 28 days later with intact virus isolated from 27 ml plasma without the adjuvant.

Antiserum 835 was obtained by immunizing a rabbit with the commercial preparation of RLV. A preparation of intact virus in a dose of 0.075 ml was injected in 4 volumes of Freund's complete adjuvant into the lymph glands. For reimmunization, 36 days later the rabbit was given an injection of 0.45 ml of the same preparation, stored at -20° C. Blood was taken from the rabbits on the 7th, 9th, and 11th days.

Mouse type-specific serum (MTS), generously provided by E. S. Ievleva, was obtained by immunizing C57BL/6 mice with the spleen cells of BALB/c mice affected with Rauscher's leukemia and exhausted by normal spleen cells from mice of the same line (BALB/c) [6]. According to the immunofluorescence test the serum possessed the type specificity of leukemias of the FMR (Friend–Moloney–Rauscher) group.

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The antigens were 30% tissue extracts in buffered 0.85% NaCl solution, pH 7.2. The tissue homogenate was disintegrated by ultrasound in the MSE-100 apparatus [1] and centrifuged for 20 min at 8000 rpm. In some cases antigens and antisera were concentrated with Lifogel (Gelman, England). Both freshly prepared extracts and extracts stored at -20° for several weeks were used. The RLV for the gel-diffusion test was treated with ether [6].

The gel-diffusion test was carried out in 1% Difco agar [2].

Immunoautoradiography. The precipitation test for immunoautoradiography was carried out in 2% Difco agar. Immunoautoradiography was performed by D. A. Él'gort by the method described previously [7]. Photographs of the reactions were prepared from the autographs.

EXPERIMENTAL RESULTS

Antibodies against the proteins of normal mouse serum (NMS), readily neutralized by 0.067 volume of the latter, were found in the antiserum 899. The antiserum 899 precipitated antigen gs-1 from a 30% extract of the spleen in Rauscher's leukemia (RLS). This precipitation line was identified with the test system for gs-1 obtained by N. V. Éngel'gardt et al. [4]. The antiserum 899 gave a second precipitation line located nearer to the antigen. The line was very weak and could be detected only with the freshly prepared extract.

Antiserum 835 did not precipitate the gs-1 antigen. This serum, in twofold concentration, gave only a weak precipitation line, difficult to analyze because of the bright halo formed by the concentrated extract, with the RLS extract in a fourfold concentration.

During analysis of the same sera by immunoautoradiography three precipitation lines were found. Antiserum 899 precipitated three antigens from the RLS extract. One antigen was identified as gs-1. The second antigen gave a precipitation line alongside gs-1 but closer to the well with the antigen. The precipitation line of the third antigen was located immediately by the well with the antigen (Fig. 1A).

Antiserum 835 and RLS extract in two- and fourfold concentration, respectively, formed only one precipitation line. The antigen precipitated by this serum was found to be identical with the second antigen precipitated by antiserum 899 (Fig. 1B). Besides in spleen extract, the antigen forming the second line was found in the plasma of mice with Rauscher's leukemia, in the commercial preparation of RLV (Fig. 1C), and in the extract of the spleen in Friend's leukemia. In Moloney leukemia the antigen was found only irregularly. The second antigen was absent in extracts of Moloney mouse sarcoma and extracts of rat sarcoma induced by mouse sarcoma virus of the Gasder strain. It was not found in the normal BALB/c mouse spleen or in Gross lymphomas in AKR mice. The extract of Gross lymphomas neutralized only antibodies against gs-1 in antiserum 899 and the antibodies against the second antigen were left intact (Fig. 1D).

The rabbit antibodies forming the second precipitation line with MTS were identified. The modification proposed by G. I. Abelev was used: rabbit antibodies were added to the invisible precipitate formed by MTS and the extract of RLS. The agar plate in which the diffusion test was carried out was placed in 0.85% NaCl, pH 7.2, for 12 h so as to remove all unreacted components from the agar. The eluate of rabbit antibodies against mouse γ-globulin was then poured into the wells containing MTS and NMS (Fig. 1E). The agar plate was again washed 18 h later for 24 h and treated with radioactive antibodies against rabbit γ-globulin. As a result of this experiment a reaction of identity was obtained between the test system for the second antigen and MTS (Fig. 1E).

On the basis of these observations the second antigen was identified as a type-specific antigen of Rauscher and Friend leukemias. The antigen forming the third precipitation line has not yet been identified.

Three antigens thus were found by the agar diffusion and immunoautoradiographic methods using rabbit antisera against intact virus in the extracts of RLS: gs-1, type-specific, and a third, unidentified antigen. Besides extracts of the spleen of animals with Rauscher and Friend leukemias, the type-specific antigen also was found in the commercial preparation of RLV. This suggests that the antigen discovered is one of the type-specific antigens of the membrane of the virus particle.

Schäfer et al. [14, 15] identified a type-specific antigen II v for Rauscher and Friend viruses and for leukemic virus v, isolated from a tissue culture. This antigen gave a reaction of partial identity with one of the group-specific antigens (II gs). These workers could not separate the antigenic determinants IIv and II gs.

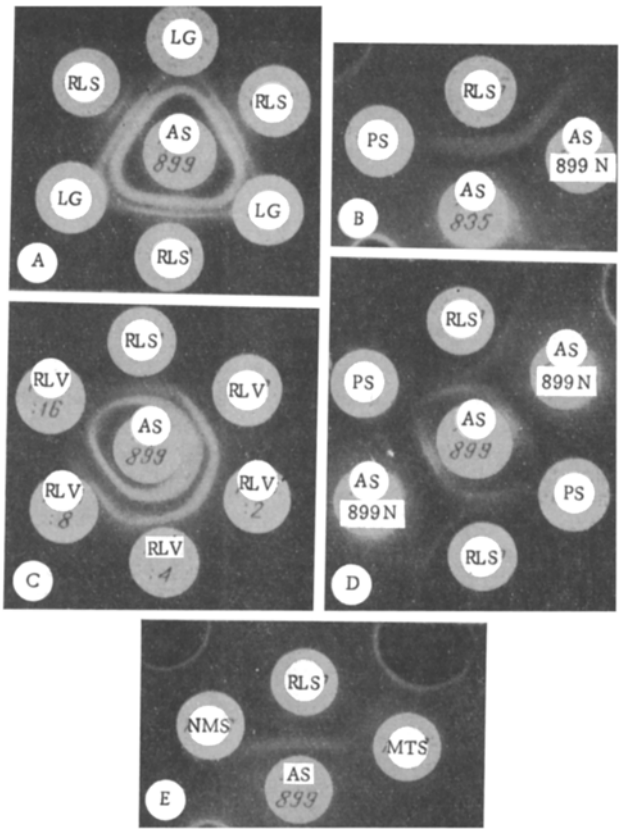

Fig. 1. Agar diffusion test for type-specific antigen (TSA) using the immunoautoradiographic technique: A) three precipitation lines detected by antiserum of rabbit immunized with intact Rauscher virus; LG) extract of Gross lymphoma; RLS) spleen extract in Rauscher's leukemia, antiserum AS 899 in the center; B) reaction of identity of two rabbit antisera precipitating type-specific antigen; AS 899N) antiserum in which antibodies against gs-1 antigen were neutralized by extract of Gross lymphoma; PS) physiological saline; C) precipitation of type-specific antigen from preparation of purified Rauscher virus (line situated closer to well containing antigen); RLV) preparation of Rauscher virus destroyed with ether; D) rabbit antiserum neutralized with extract of Gross lymphoma (AS 899N) continues to precipitate type-specific antigen from RLS. This antiserum works with AS 899N, neutralized by an excess of gs-1 antigen; E) reaction of identity between rabbit antiserum and mouse specific antisera precipitating type-specific antigen.

The present writer evidently obtained antibodies against another type-specific antigen unconnected with the determinants of the gs-antigens. This is shown by the complete absence of reaction with extract of Gross lymphomas and by adsorption of the extracts of antibodies against antigen gs-1 in the antiserum 899. In that case antibodies against the type-specific antigen were not neutralized.

The type-specific antigen identified by the writer was evidently present in a smaller amount in Moloney virus than in RLV, for it was detected irregularly in Moloney leukemia. The antigen was not found in extracts of Moloney mouse sarcomas, in agreement with the observations of Fischinger et al. [9] of the absence of antigen IIv in $S^{+}L^{-}$ cells. The antigen was not found in rat sarcomas induced by mouse sarcoma virus of the Gasder strain.

The identification of rabbit precipitating antibodies with the MTS antibodies also indicates the type-specific nature of the second antigen. However, the question of the location of this antigen in the membrane of the virus particle or in the cell membrane can be finally settled only by electron-microscopic investigation with monospecific antibodies.

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LITERATURE CITED

1. G. I. Abelev, E. S. Ievleva, and N. V. Éngel'gardt, Biokhimiya, 33, 509 (1968).
2. A. I. Gusev and V. S. Tsvetkov, Lab. Delo, No. 2, 43 (1961).
3. N. A. Dorfman, V. I. Stepina, and E. S. Ievleva, Vestn. Akad. Med. Nauk SSSR, No. 4, 50 (1973).
4. E. S. Ievleva, N. V. Éngel'gardt, and G. I. Abelev, Byull. Éksperim. Biol. i Med., No. 10, 73 (1969).
5. V. S. Tsvetkov, in: Immunochemical Analysis [in Russian], Moscow (1968), p. 120.
6. N. V. Éngel'gardt, G. I. Abelev, and E. S. Ievleva, Vopr. Onkol., No. 10, 75 (1969).
7. G. I. Abelev and D. A. Él'gort (Elgort), Internat. J. Cancer, 6, 145 (1970).
8. R. J. Eckner and R. A. Steeves, J. Exp. Med., 136, 832 (1972).
9. P. J. Fischinger, W. Schäfer, and E. Seijert, Virology, 47, 229 (1972).
10. R. B. Goudie, C. H. W. Horne, and P. C. Wilkinson, Lancet, 3, 1224 (1966).
11. R. J. Huebner and L. J. Todaro, Proc. Nat. Acad. Sci. (Washington), 64, 1087 (1969).
12. B. Micheel and B. Bierwolf, Exp. Cell Res., 54, 268 (1969).
13. L. J. Old, E. A. Boyes, G. Geering, et al., Cancer Res., 28, 1288
14. W. Schäfer, P. J. Fischinger, J. Lange, et al., Virology, 47, 197 (1972).
15. W. Schäfer, J. Lange, P. J. Fischinger, et al., Virology, 47, 210 (1972).