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SHORT COMMUNICATION

RABBIT ANTISERUM AGAINST AUSTRALIA ANTIGEN

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Although there are many reports concerning Australia antigen which was first described by Blumberg in 1966, there is only one report concerning antigenicity of Australia antigen. It has been described by Blumberg (Melartin and Blumberg 1966, Levene and Blumberg 1969), who used whole human serum containing Australia antigen and produced antiserum aganinst Australia antigen in a rabbit.

In Japan, physician have recently tended to avoid use of blood containing Australia antigen for blood transfusion, so in near future researchers will not use antiserum of human origin. Thus it has become necessary to produce antiserum against Australia antigen in rabbits.

This paper reports experiments on rabbit antibodies to Australia antigen evoked by an immune complex of Australia antigen in human serum and homologous human antibodies. For preparation of the immune complex, human sera containing the antibodies were obtained in the Osaka area and sera containing Australia antigen were provided by Dr. Okochi. The precipitation line of our system fused with that of a reference system provided by Dr. Okochi of Tokyo University, which had been proved to be identical with Blumberg's system (Levene and Blumberg 1969, Okochi and Mrakami 1968). Ten ml of human antiserum against Australia antigen and 10 ml of human serum containing Australia antigen were mixed, and incubated at 30 C for 1 hr. The mixture was then stood at 4 C for 5 days. The precipitate was centrifuged for 30 min at 28,000 rev/min in the SW 30 rotor of a Beckman L Ultracentrifuge. The resulting precipitate was washed five times with cold saline and then suspended in 4 ml of physiological saline. This suspension was used for immunization, and was also examined by electronmicroscopy. Two ml of the suspension of the immune complex described above were mixed with Freund's complete adjuvant, and injected into rabbits intramuscularly. The first injection was followed by a similar one 2 weeks later. After 3 weeks, 20 ml of blood were taken. The sera contained antibodies to human immunoglobulins, so these were absorbed with gels of normal human sera containing no Australia antigen prepared with glutaraldehyde by the method of Avrameas (1969). The absorption treatment was repeated 10 times until no precipitation line was found against normal human sera. In addition material in a precipitation line in agar gel diffusion between human antiserum and human serum containing Australia antigen was used for immunization of rabbits. This gave similar results, but the results described in this article

were obtained by immunization with the antigen prepared by the first method described above.

Immunodiffusion (Ouchterlony's technique) and immunoelectrophoresis were carried out in 0.8% agarose in veronal-acetate buffer, pH 8.6, μ=0.05. The precipitation line formed with this absorbed rabbit antiserum against human sera containing Australia antigen (AU+) was compared with that of human antibodies. A precipitation line demonstrating identity without a spur was obtained, as shown in Fig. 1, and no precipitation line was given with normal human serum containing no Australia antigen (AU−). On immunoelectrophoresis, it gave a single precipitation line against 47 human AU+ sera in the region of α- and β-globulin. A single line was also detected in the same position with human antibodies. However, two human AU+ sera, gave two additional lines with absorbed rabbit antiserum, in the regions of α-globulin and γ-globulin, respectively. The relationship between these additional lines and the main line is unknown, but these additional lines were not shown by human antibodies. On gel diffusion these two sera each gave a single line against rabbit antiserum. However, these sera formed an opalescent zone around the antigen well, so it is uncertain how many precipitation lines were formed. The AU+ human sera, provided by Dr. Okochi and used for preparing the immunizing antigen also gave a single line on immunoelectrophoresis with this absorbed rabbit antiserum.

The counterimmunoelectrophoresis technique, recently described by Gocke (1970), was applied to this rabbit antiserum. A very fine precipitation line was obtained against AU+ human serum but none against AU− human serum. The two AU+ human sera, which gave two additional lines on immunoelectrophoresis with rabbit antiserum, gave a strong but slightly broader line, and the remaining AU+ sera gave a single line, as shown in Fig. 3.

Although our antiserum was weak and early

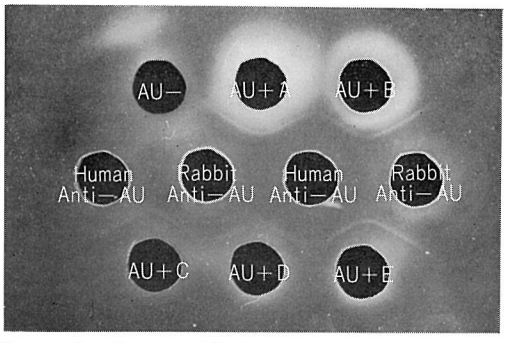

FIGURE 1. *Immunodiffusion picture comparing human antiserum with rabbit antiserum.*

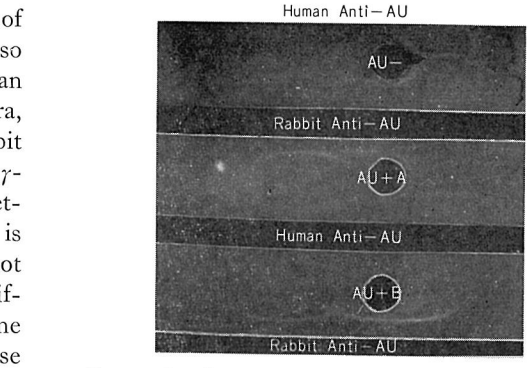

FIGURE 2. *Immunoelectrophoresis comparing human antiserum with rabbit antiserum.*

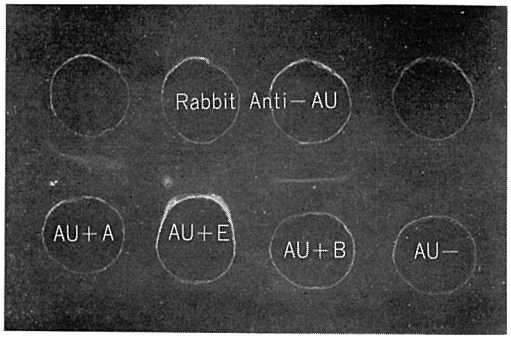

FIGURE 3. "*Counterimmunoelectrophoresis*" *with rabbit antiserum.*

AU+A and AU+B: Individual sera containing Australia antigen obtained in the Osaka area.

AU+C, AU+D and AU+E: Individual sera containing Australia antigen provided by Dr. Okochi.

one to come to conclude, certainly has antigenicity against rabbits, and our results confirm Blumberg's findings (Melartin and Blumberg 1966, Levene and Blumberg 1969).

There are serveral advantages in using an immune complex instead of whole serum containing Australia antigen. First, the immune complex contains less antigenic protein than whole serum containing Australia antigen, and hence absorption of antibodies to other serum components is easier. Secondly, it is very easy to prepare antigen for immunization.

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