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Mouse Immunoglobulin Allotypes: Detection with Rabbit Antiserums

Abstract. *An antiserum with allotypic specificity for mouse γ_{2a}-globulins was prepared in a rabbit by injection of 7S γ_2-globulin of $C_{57}BL/6$ mice. The antibody reacted with an isoantigen in the 7S γ_2-globulins of normal serum of mouse strains belonging to the Ig-1^b allotype class ($C_{57}BL/6$, $C_{57}BL/10$, and SJL strains). No precipitin reaction was observed with serum from 18 other inbred mice strains representing other Ig-1 allotype classes.*

Mouse immunoglobulin allotypes were first described by Kelus and Moor-Jankowski (*1*). Serums of Balb/c mice injected into $C_{57}BL$ mice induced an antibody response specific for a Balb/c globulin. Others (*2–5*) have approached the problem similarly, using various combinations of mouse donors and recipients. The mouse immunoglobulin allotype is a suitable model for study because: (i) a large number of inbred strains are available, and (ii) the protein containing the isoantigen is relatively easy to isolate. However, the mouse is not a convenient animal for preparing antiserums. I now describe a rabbit antibody with an allotypic specificity for an isoantigen of the MuA_2 (*2*) [Asa^2 (*4*), Ig-1^b (*5*)] group.

A single New Zealand white rabbit was injected subcutaneously on day 0 and day 7 with a 7*S* γ_2-globulin preparation (*6*) obtained by the Pevikon method of block electrophoresis (*7*) of normal $C_{57}BL/6$ serum. The most cathodal globulin fraction was emulsified in Freund's adjuvant (Difco) containing 8 mg of *Mycobacterium tuberculosis* per milliliter. Rabbit serum obtained on day 31 was examined by agar diffusion techniques (*8*). This antiserum produced two distinct 7*S* γ_2 precipitin lines on the immunoelectrophoresis plate in the reaction with $C_{57}BL/6$ serum, but only one precipitin line when CBA serum was used as antigen (Fig. 1, trough *b*). The following technique was used to evaluate the relation of CBA serum to the aforesaid two precipitin lines of 7*S* γ_2 seen with $C_{57}BL/6$ antigen. After electrophoresis of the $C_{57}BL/6$ serum, coincident with charging the troughs with antibody, small wells were made at appropriate sites and filled with CBA serum. The outer precipitin line was deviated by the CBA serum, but the inner line was not affected (Fig. 1, trough *d*). Serums from 19 other inbred strains (Jackson Memorial Laboratory, Bar Harbor, Maine) were similarly evaluated and only $C_{57}BL/6$, $C_{57}BL/10$, and SJL strains deviated the inner line whereas those from A/J, A/HeJ, AKR/J, DBA/1J, DBA/2J, $C_{57}BR/cd$, C_{58}/J, $C_{57}L/J$, C_3H/HeJ, C_3HeB/FeJ, CE/J, MA/J, RF/J, ST/J, SWR/J, 129/J, Balb/c, and CBA affected the outer line exclusively. Similarly two myeloma proteins (LPC-1 and MPC-31) (*9*) obtained from Balb/c mice representing respectively, the 7*S* γ_{2a}- and 7*S* γ_{2b}-immunoglobulin subclasses (*10*) did not deviate the inner line. Both of these precipitin lines seen on immunoelectrophoresis represent immunoglobulins, because specific binding of radioactive antigen was observed by autoradiography (Fig. 2).

Two milliliters of the rabbit antiserum to 7*S* γ_2-globulin of $C_{57}BL/6$ mice were absorbed successively with 0.05-ml portions of CBA serum (40 : 1 ratio). After incubation overnight at 4°C, the resulting precipitate was removed by centrifugation, and 0.25 ml of supernatant was saved for testing prior to the addition of another 0.05 ml of CBA serum. A control antiserum was treated in a similar fashion except that saline was added. After the second absorption with CBA serum, the mixture of antiserum and CBA serum contained excess of antigen common to the CBA 7*S* γ_2-globulins (Fig. 3, top wells). However, the antiserum, even after three more absorptions, still formed a precipitin line with $C_{57}BL/6$ serums (Fig. 3, bottom wells). Figure 1 (troughs *a* and *c*) shows antiserum absorbed five times with CBA compared, on immunoelectrophoresis, with control antiserum (Fig. 1, troughs *b* and *d*).

The excess CBA serum in the absorbed antiserum forms a precipitin line with the control antiserum. This five-times-CBA-absorbed antiserum was tested by agar diffusion against the 21 serum samples from the inbred mice and the two myeloma proteins. A precipitin reaction was observed only with $C_{57}BL/6$, $C_{57}BL/10$, and SJL inbred serums, and these precipitin lines fused in a reaction of identity (*11*). A

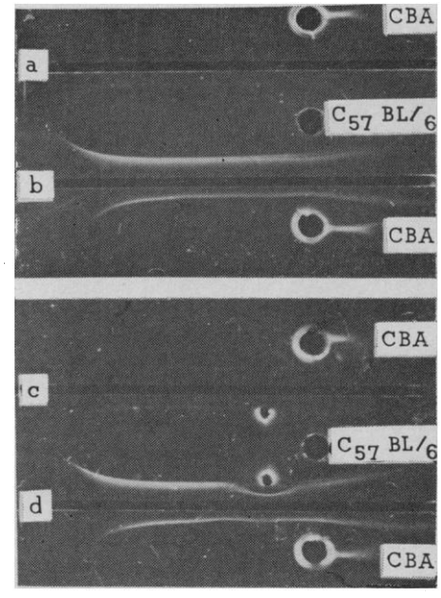

Fig. 1. Immunoelectrophoretic patterns of CBA and $C_{57}BL/6$ serums with rabbit antiserum to $C_{57}BL/6$ 7*S* γ_2-globulin absorbed with CBA serum and saline. Addition of CBA serum to the small wells shows that the external line contains common CBA antigens, whereas the internal line does not. Troughs *a* and *c* contain rabbit antibody to $C_{57}BL/6$ 7*S* γ_2 absorbed five times with CBA. Troughs *b* and *d* contain rabbit antibody to $C_{57}BL/6$ 7*S* γ_2 that had been absorbed five times with saline.

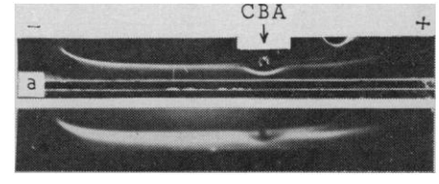

Fig. 2. Immunoelectrophoresis plate (top) and respective hen egg albumin (HEA) I^{131} autoradiograph (bottom) of SJL serum containing antibody to HEA. Antigen-binding capacity can be seen in both precipitin lines. Autoradiography as follows: the developed immunoelectrophoresis slide was washed for 24 hours in saline and photographed; the troughs were then charged with HEA I^{131}. After 24 hours, the slide was washed with repeated changes of saline for 3 days, dried, stained, and affixed to Kodak projection slide plates for 1 week.

pool of serums from RML mice (a random-bred Swiss strain maintained as a closed colony at the Rocky Mountain Laboratory for the past 28 years) also produced a precipitin line with the absorbed antiserums. Addition of $C_{57}BL/6$ serum to the antiserum to $C_{57}BL/6$ 7*S* γ_2-globulin at a 1:20 ratio neutralized all detectable antibody whereas specific antibodies to $C_{57}BL/6$ were still detectable even after addition of CBA serum in a 1 : 1 ratio. The precipitate resulting from the addition of CBA serum to the antibody to $C_{57}BL/6$ 7*S* γ_2-globulin was washed five times, dissolved at *p*H 3 by addition of acetic acid, and emulsified in complete Freund's adjuvant. This preparation injected into two rabbits induced an antibody response to 7*S* γ_2-globulin, but no antibody specific for $C_{57}BL/6$ could be detected.

The unabsorbed rabbit antiserum to $C_{57}BL/6$ 7*S* γ_2-globulin reacted on Ouchterlony plates with myeloma proteins of the 7*S* γ_{2a} and 7*S* γ_{2b} subclasses. Absorption with 7*S* γ_{2a} myeloma protein removed all antibody activity; however, absorption with 7*S* γ_{2b} left a small amount of antibody activity for the 7*S* γ_{2a} myeloma protein. Therefore, the unabsorbed antiserum contained antibodies with specificities directed toward the common 7*S* γ_2 antigen, the $C_{57}BL/6$ isoantigen, and also the 7*S* γ_{2a} antigen. Presumably the outer line on immunoelectrophoresis with $C_{57}BL/6$ serum represents a reaction with the common 7*S* γ_2 antigens, and the inner line a reaction with the $C_{57}BL/6$ isoantigen which should be on the F_c piece (fragment c) of 7*S* γ_{2a} where the antigen controlled by the allele Ig-1^b has been shown to reside (*12*). Antiserums against 7*S* γ_{2a}- and 7*S* γ_{2b}-globulins were prepared in rabbits by immunization with 7*S* γ_{2a} and 7*S* γ_{2b} myeloma proteins and made specific by cross absorptions.

The precipitin lines that were made by these antiserums were compared with that from the specific reaction of $C_{57}BL/6$ with antibody to $C_{57}BL/6$ (Fig. 4). The precipitin line due to the reaction of $C_{57}BL/6$ with antibody to $C_{57}BL/6$ crossed the precipitin line due to 7*S* γ_{2b} and antibody to 7*S* γ_{2b}, but it did not cross the precipitin line due to 7*S* γ_{2a} and antibody to 7*S* γ_{2a}. This finding indicated that the 7*S* γ_{2a} determinant and $C_{57}BL/6$ isoantigen determinant were present on the same

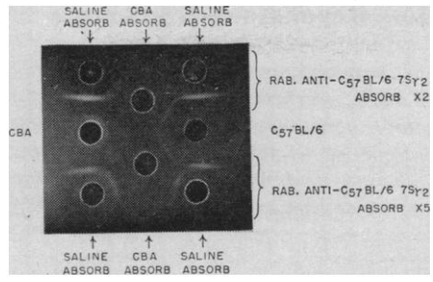

Fig. 3. Absorption of rabbit antibody to $C_{57}BL/6$ 7*S* γ_2-globulin with CBA serum and saline. Two additions of CBA serum to the antiserum removed all antibody specific for CBA antigen (top well). Specific antiserum to $C_{57}BL/6$ was not affected by three subsequent CBA absorptions (bottom well).

molecule. Figure 4 also shows that the precipitin line due to 7*S* γ_{2a} myeloma protein and antibody to 7*S* γ_{2a} myeloma protein does not fuse completely with the precipitin line formed by $C_{57}BL/6$ and antibody to 7*S* γ_{2a} myeloma protein. A specific antibody to Balb/c found in this antiserum and also in another rabbit antiserum to 7*S* γ_{2a} myeloma protein, after absorption with $C_{57}BL/6$ serums, provides another example of rabbit antiserums with allotypic specificity.

A rabbit antibody with allotypic specificity for mouse immunoglobulins is of some practical importance, for example, the rabbit represents a dependable source for large quantities of high-

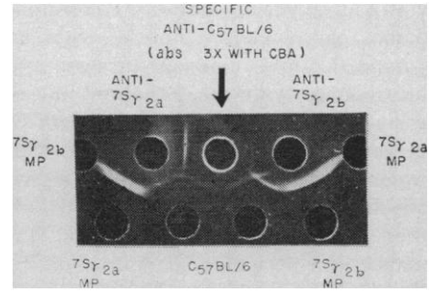

Fig. 4. Double diffusion test showing the relation between the precipitin line produced by $C_{57}BL/6$ and antibody to the isoantigen of $C_{57}BL/6$ and the precipitin lines produced by 7*S* γ_{2a}- and 7*S* γ_{2b}-globulins and specific 7*S* γ_{2a} and 7*S* γ_{2b} antibody. The precipitin line resulting from action of antiserum to $C_{57}BL/6$ isoantigen crosses the precipitin line formed by 7*S* γ_{2b}-globulin and antibody to 7*S* γ_{2b}, but does not cross the precipitin line formed by 7*S* γ_{2a}-globulin and antibody to 7*S* γ_{2a}. Also note that the precipitin line resulting from 7*S* γ_{2a}-globulin and antibody to 7*S* γ_{2a} does not fuse completely with the precipitin line formed by $C_{57}BL/6$ and antibody to 7*S* γ_{2a}.

titer antibody. Also rabbit antiserum provides another means for recognition of allotypes and for correlating the allotype data obtained from the mouse. This allotypic antibody may be produced with some consistency. Five rabbits were subsequently injected with the same $C_{57}BL/6$ globulin preparation emulsified in complete Freund's adjuvant and antiserums collected 19 days later.

A similar allotypic antibody to $C_{57}BL/6$ isoantigen was detected in serums from four rabbits. The immune response of the fifth rabbit was generally weak, the serum showed little antibody to 7*S* γ_2, and the isoantigen antibody could not be detected. The antibody to the allotype in this study is probably directed to the MuA_2 (Asa^2, Ig-1^b) antigen because (i) a positive precipitin reaction was obtained only with serums from inbred strains representing the Ig-1^b class, and all tested members of this class produced a positive reaction; and (ii) the allotypic antigen detected by these antiserums was located in the 7*S* γ_{2a}-globulin, the 7*S* γ_2-globulin subclass which has been shown to carry the Ig-1 isoantigen (*10*). Thus, multiple precipitin lines on agar diffusion may be an expression of allotypic specificity and not a result of antibody primarily directed to different types or classes of globulins.

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