

Plaque Formation in Agar by Single Antibody-Producing Cells

Abstract. *Distinct plaques, each of which is due to the release of hemolysin by a single antibody-forming cell, are revealed by complement after incubation, in an agar layer, of a mixture of sheep red cells and lymphoid cells from a rabbit immunized with sheep red cells.*

We have developed a simple technique for scoring individual antibody-forming cells among a mixed cell population. The following experimental example describes the procedure.

From a rabbit that had received three injections, each of 5 times 10^6 sheep red cell stromata, in the footpads during the preceding three weeks, a popliteal lymph node was removed and its contents of cells teased out into tissue culture medium containing no serum (1). Microscopic examination of the cell suspension obtained after washing by three centrifugations and resuspensions in this medium in the cold showed the presence of non-aggregated lymphoid cells of various types. One million of these cells in 0.1 ml as well as 200 million sheep red cells in 0.1 ml were added to 2 ml of a 0.7-percent Difco-agar solution in the culture medium that was kept fluid at 45°C, and the mixture was poured onto a supporting 1.4-percent agar bottom layer in a petri dish so as to form a thin, semisolid top layer. After incubation at 37°C for 1 hour this layer was covered with 1.5 ml of complement (guinea pig serum 1:5). Further incubation for 15 minutes re-

vealed about 100 clear plaques of about 0.25 mm diameter that stood out sharply against the uniformly red background.

Experiments of this type with rabbit lymph node cells as well as with mouse spleen cells have shown that the number of plaques obtained is proportional to the number of lymphoid cells plated. This suggests that each plaque is due to the activity of an individual cell. Microscopically, a plaque shows up as a circular hemolytic clearance in a field of closely scattered red cells, and in the center of most plaques a lymphoid cell is seen which presumably is the cell that released the sensitizing antibody (Fig. 1). Larger plaque sizes are obtained by using a lower red cell concentration, though this

diminishes the color contrast. Plaque formation did not occur in the presence of 0.01 molar potassium cyanide.

By using red cells coated with other antigenic determinants, the technique may be extended to other antigen-antibody systems, whereas multiple antibody production by individual cells could be studied by using mixed red cells. We are now investigating the question of the efficiency of plating.

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Reference

1. G. A. Fischer, *Ann. N.Y. Acad. Sci.* **76**(3), 673 (1958).

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Antibody Quality after Sequential Immunization with Related Antigens

Abstract. *The response to the second antigen of a sequence of two related antigens appears to consist of two qualitatively different antibody populations. Antibodies specific for the second antigen are of primary-response quality while the antibodies which cross-react with the first antigen are of secondary-response quality.*

The substantial body of information demonstrating qualitative differences between antibodies produced after a first and second injection of the same antigen (1, 2) makes it necessary to re-evaluate interpretations of antibody responses based solely on quantitative data. One such situation is the response to sequential immunization with two related antigens. The conclusions based on past studies range from (i) anamnestic (secondary) responses to both antigens (3) to (ii) a response directed mainly against the first antigen of the sequence (4) to (iii) a response directed mainly against the cross-reacting determinants of the two antigens along with a primary response specific for the second antigen and a small amount of antibody specific for the first antigen (5). We have been able to clarify this problem partially by studying antibody quality, as measured by the rate of dissociation of labeled antigen from antigen-antibody complexes.

White Rock male chickens, 10 weeks old, obtained from a commercial breeder were injected with 40 mg of one antigen, either crystalline bovine serum albumin (BSA) or human serum albumin (HSA), followed 45 days later by 40 mg of the same or the other antigen. In the chicken approximately 10 percent of the antibodies produced

against either antigen can be precipitated with the other antigen (5). Serum samples were obtained 6, 8, and 15 days after each injection as well as 43 days after the first injection. In order to obtain specific antibodies, the cross-reacting activity was absorbed out by incubating the serum with a large excess of the appropriate antigen for several days. Our technique is a modification of that described previously by Farr (2) and Talmage (6). Serum which had been diluted to bind 20 to

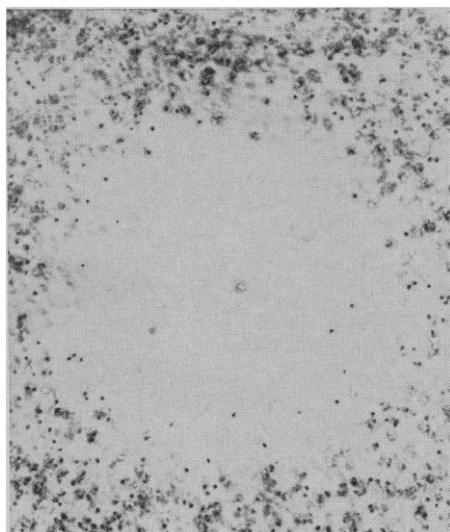


Fig. 1. Rabbit lymph node cell producing a plaque.

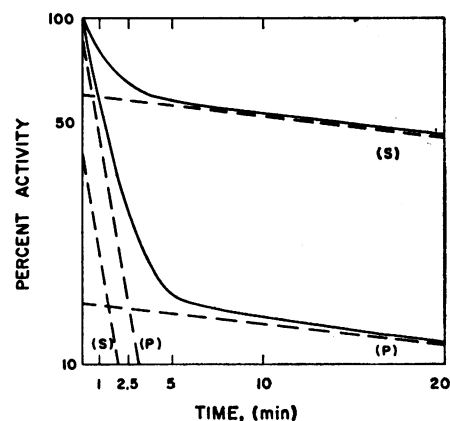


Fig. 1. Dissociation curves typical of antisera obtained 8 days after one (P) and two (S) injections of either BSA or HSA. Dotted lines represent the two components of each curve.