Bacteria as an Indicator of Formation of Antibodies by Single Spleen Cells in Agar

Abstract. *Bacteria can be used to measure the formation of specific antibody by suspensions of single spleen cells. The technique, based on localized immune lysis (plaque formation) in agar, permits assays on the kinetics of increase in the number of cells forming bactericidal antibody in the spleen of immunized mice. The procedure also is useful for testing nonspecific stimulation of antibody synthesis and for analyzing spleen cell populations from donors that have been immunized simultaneously with bacterial and red blood cell antigens.*

Jerne's technique for the quantitative assay of antibody production by suspensions of single spleen cells in agar (*1*) has been very useful for immunological studies. The technique depends on the ability of specific antibodies to lyse red blood cells, from a heterologous donor, in the presence of components of the complement (C′) system. Consequently, plaques are produced when spleen cells forming the required specific antibody are plated in agar with heterologous erythrocytes and C′. This method has limitations since it can be used only with red blood cells or red blood cells coated with bacterial antigens (*2*). The same basic technique, however, can also be used with Gram-negative bacteria which are killed in the presence of specific antibody and C′ (*3*).

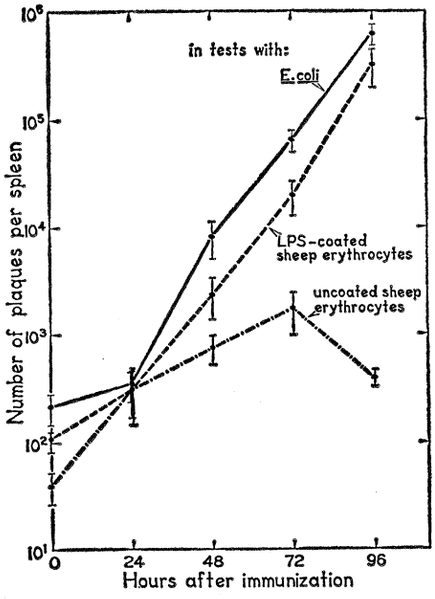

Fig. 1. Average (± S.E.) of plaque-forming spleen cells in AKR mice immunized with 10^9 heat-killed *Escherichia coli* O127. (The zero-hour figures also represent the normal "background" of spleen cells forming hemolysins and bactericidal antibody, respectively.)

Mice (AKR strain, 8 to 14 weeks old) were immunized with *Escherichia coli* O127 (10^9 cells, heat-killed at 65°C for 1 hour). Mice were killed, and their spleens were removed aseptically at various intervals thereafter. A suspension of single spleen cells was prepared as described (*1*) except that Earle's balanced salt solution (2 ml per spleen), free of calcium and magnesium ions, was substituted for Eagle's medium. The suspension was filtered through sterile, fine-mesh, nylon gauze and serially diluted in the suspending medium; the actual dilution used depended on the anticipated proportion of antibody-forming cells (*4*). Equal volumes of sterile, double-strength Eagle's minimal essential medium (MEM, free of phenol red) and 1.4 percent Noble's agar (Difco) were mixed at 45°C; 5 ml of this MEM agar was poured as a basal layer into petri dishes (100 mm in diameter). To another portion (2 ml) of MEM agar, supplemented with a sterile aqueous solution of diethylaminoethyl-dextran (0.5 mg per ml agar), were added: 0.1 ml of a culture of *E. coli* O127 (approx. 1×10^9 organisms/ml) grown (with shaking) for 12 to 18 hours in tryptose broth and 0.2 ml of the spleen cell suspension described above. This mixture was poured over the basal layer, permitted to harden, and incubated at 37°C for 1 hour. Subsequently, 1.5 ml of a 1:1 dilution of guinea pig complement and acetate–barbiturate (AB) buffer (*5*) was spread over the surface of the plates; these were then kept at 4°C for 1 hour (to permit diffusion of C′ in the absence of bacterial growth) and at 37°C for 12 to 18 hours. They were finally examined for plaques in the layer of confluent bacterial growth.

Spleens removed at daily intervals from donors immunized with *E. coli* show similar increases in the number of plaques that develop in assays with *E. coli* and in assays with sheep red blood cells coated with *E. coli* O127 lipopolysaccharide (Difco) (Fig. 1). However, the coated red cells also react with antibody against sheep red cells, and the number of spleen cells forming such hemolysins (revealed by tests with uncoated red cells) also increases after immunization with *E. coli* (Fig. 1). Consequently, the use of coated red cells in assays for antibodies directed only against antigens of Gram-negative bacteria is really much more inefficient than the use of whole bacterial cells, particularly during the early stages of the immune response. The increase in the number of cells forming antibody to sheep red cells after immunization of the spleen donor with *E. coli* can be attributed to an endotoxin effect (*6*, *7*). Immunization of the spleen donors with sheep red blood cells does not similarly elevate the "background" (that is, the normally present) small number of spleen cells capable of forming antibodies against *E. coli*.

Since it is now possible to use both bacteria and red blood cells as indicators of antibody formation by single spleen cells, studies on possible competitive interactions after immunization with these antigens have become feasible. Initial tests have revealed that the kinetics of spleen responses to bacterial and erythrocyte antigens are not altered significantly when individual spleen donors are immunized simultaneously with relatively large amounts of both antigens. Also, as in the case of hemolysin production (*7*), the administration of oligodeoxyribonucleotides in conjunction with bacteria significantly increases the number of spleen cells that form antibodies to *E. coli* within 48 hours after immunization.

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References and Notes

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