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Plaques of Hemagglutination Inhibition by Individual Spleen Cells from Rabbits Immunized with Influenza Viruses

B. H. PETERSEN, Z. BRAHMI, J. S. INGRAHAM, AND A. S. LEVINE
Department of Microbiology, Indiana University School of Medicine, Indianapolis, Indiana 46202

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Spleen cells from rabbits immunized with influenza virus cause inhibition of agglutination or hemolysis or both in a plaque assay test against virus-treated avian RBC.

Hemolytic plaque techniques detect the antibody produced by individual antibody-forming cells against various species of red blood cells (RBC; 1-3) or even against anti-RBC globulin adsorbed to RBC (4). Since influenza viruses adsorb spontaneously to RBC (5), it was suggested that cells making antiviral antibody might also be detected by a hemolytic plaque technique.

Rabbits were injected in the ear vein on day 0, day 1 or 2, and day 12 or 14 with 3 ml each of partially purified virus. Fractional centrifugation or adsorption and elution of virus from chicken erythrocytes (CRBC) were the methods used to prepare virus harvested from infected allantoic fluid of 12-day-old chicken eggs which had been inoculated 48 hr earlier with 10^3 ID_{50} of strains WS or Lee of influenza virus. The viral hemagglutination (HA) titers of the inocula used to immunize rabbits were approximately 8,000 to 12,000 per ml as determined by the Salk pattern technique. Suspensions of spleen cells obtained 3 days after the final injection were examined by our microchamber plaque assay technique (6-7) on a special plaque assay slide (Bellco Glass Co., Vineland, New Jersey; antibody plaque assay slide, catalog no. 1955; 1 by 3 inch, bearing two rectangles of ceramic paint, 19 by 20 mm by 30 μm). The assay mixture contained spleen cells, RBC with adsorbed virus, and guinea pig complement. Because of the absence of any thickening agent in the assay system, the cells effectively formed a monolayer as they settled to the surface of the slide. The assay slides were incubated at 37 C for 70 min, and the plaques were scored with the aid of a microscope fitted with a 2.5 $\times$ objective and making use of a type of dark-field illumination as described previously (3).

At the end of the incubation at 37 C, there were hemolytic plaques in a matrix of uniformly dispersed RBC. At room temperature, the RBC gradually agglutinated in those chambers containing virus but remained uniformly dispersed in controls without virus. After 1 to 2 hr at room temperature, the RBC layer in chambers containing both virus and immune spleen cells was agglutinated throughout except for discrete, circular areas in which the RBC remained uniformly dispersed (Fig. 1). These areas were about the size of the hemolytic plaques, and we interpreted this new type of plaque as the result of localized specific inhibition of the viral HA by antiviral antibodies secreted by the immune spleen cells. These HA inhibition plaques occurred in the presence or absence of complement.

Using either hemolysis or HA-inhibition plaque formation as the criteria of antibody formation, we examined the plaque-forming-cell (PFC) response (Table 1). Spleen cells from rabbits

TABLE 1. *Antiviral PFC response*

Rabbit	Immunizing antigen	Assay cells	PFC[a]	Net specific PFC[a]
102	None	WS-CRBC	4	
103	WS	CRBC	161	
103	WS	WS-CRBC[b]	223	62
103	WS	PRBC	11	
103	WS	WS-PRBC	108	97
26	WS	PRBC	5	
26	WS	WS-PRBC	167	162
29	WS	PRBC	13	
29	WS	WS-PRBC	45	32
104	Lee	CRBC	566	
104	Lee	WS-CRBC	570	
104	Lee	Lee-CRBC	740	170

[a] Values express PFC/10^6.
[b] WS-CRBC, CRBC treated with influenza virus strain WS.

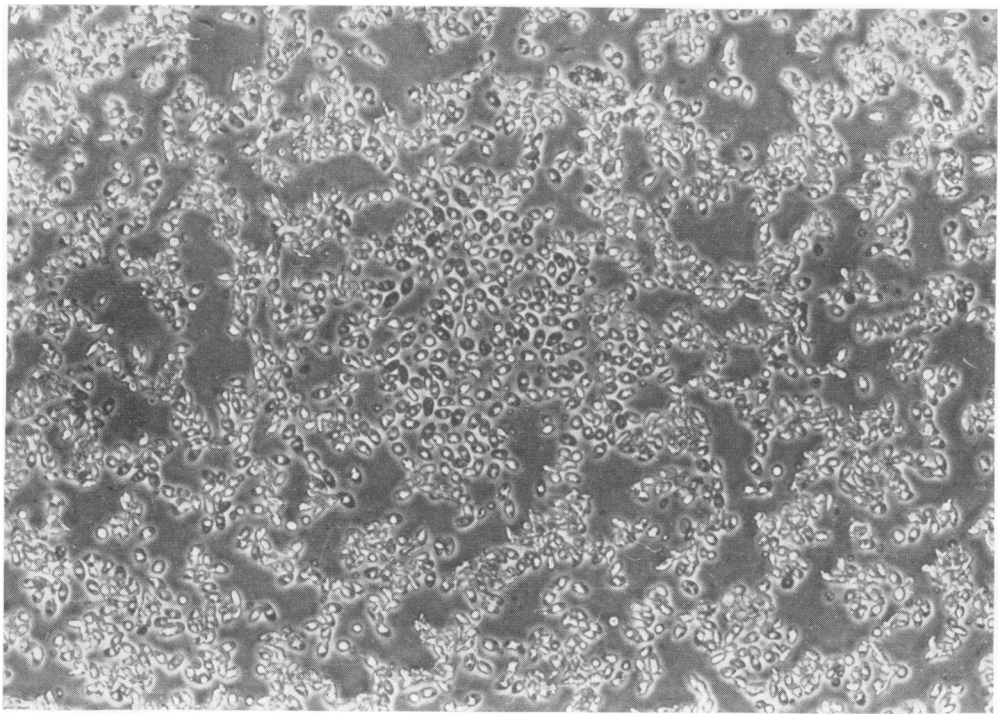

FIG. 1. *HA inhibition plaque taken with × 10 phase contrast objective. × 100. The contrast between the uniformly dispersed PRBC in the plaque and the coarse aggregates in the background is quite distinct, but this difference is even more striking in color. One can then see a brilliant orange mottling of the clumps where the agglutinated RBC overlap each other but not at all in the plaque area.*

immunized with influenza virus strains WS or Lee produced plaques on RBC treated with these viruses. Demonstration of specific plaques with CRBC as the assay cell was complicated by cross-reaction between CRBC and the virus preparation (rabbits 103 and 104). Substitution of pigeon RBC (PRBC) for CRBC reduced the cross-reactivity, allowing observation of plaques many-fold in excess of the background PRBC plaques (Table 1).

Data from rabbit 104 immunized with influenza virus strain Lee and tested on influenza virus strain WS-coated CRBC suggested that the cells were making a type-specific antiviral antibody.

After absorption with CRBC, autopsy sera from these rabbits were examined for antiviral activity by HA inhibition. As might be expected there was considerable cross-reaction between the WS and Lee antisera, but rabbits 103, 26, and 29 immunized with strain WS gave inhibition titers eightfold higher against strain WS than against strain Lee, whereas rabbit 104 immunized with strain Lee gave a fourfold higher inhibition against strain Lee than against strain WS.

These results support the hypothesis that it may be possible to use virus-coated RBC to detect antiviral antibody produced by single cells.

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