Reactions of Intracellular Crystals of Foot-and-Mouth Disease Virus with Ferritin-tagged Antibody

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SUMMARY

Crystals of intracellular foot-and-mouth disease virus, type A1, were reacted with ferritin conjugated to an A1 antibody, an O2 antibody, and normal γ-globulin as well as unconjugated ferritin. Specific tagging was demonstrated with both the full and ‘empty’ capsid crystals with type A1 virus and anti-A1 globulin. Anti-O2 ferritin-conjugated globulin, normal ferritin-conjugated γ-globulin and unconjugated ferritin did not attach to the A1 virus and showed only minimal reaction with tissue fragments and debris. Immunodiffusion in agar gel was used to demonstrate the specificity of the reagents.

INTRODUCTION

We have already reported on the intracellular formation of foot-and-mouth disease virus (FMDV) crystals with type A, strain 119, and type A1 viruses as well as the lack of such crystal formation by virus of types O2 or C3 (Breese & Graves, 1966; Breese & Graves, 1967). Crystals of both complete and ‘empty’ particles have been observed (Breese, 1968; Graves, Cowan & Trautman, 1968). The present report concerns the interaction of intracellular FMDV crystals and ferritin-tagged antibody. Various combinations of virus and ferritin-tagged antibody were examined, to try to locate early formation of virus in cells.

METHODS

Virus and tissue cultures. The FMDV (A1) strain of virus, recently isolated in Argentina, was stored as the sixth bovine kidney tissue culture passage from the original tongue tissue. This stock virus was passed twice more in primary swine kidney cells and stored as tissue-culture fluid. This virus passage (BK*PK3) and the next (BK*PK3) were used to infect cells for these experiments. Primary swine-kidney-cell cultures in 4-oz prescription bottles were used after preparation by trypsinization of minced kidney cortex.

γ-Globulin. γ-Globulin was separated by sodium sulphate precipitation and subsequent dialysis against water and phosphate-buffered saline (Rifkind, Hsu & Morgan, 1964) from the sera of guinea pigs hyperimmunized (Cowan & Graves, 1966) against FMDV (A1) and (O2). γ-Globulin from untreated guinea pigs was also prepared in a similar manner for conjugation with ferritin.

Ferritin conjugation. A detailed outline has recently been prepared by K. C. Hsu
(private communication) of methods based on those of Singer (1959) and modifications by Rifkind et al. (1964). Horse-spleen ferritin, once crystallized (Pentex, Inc., Kankanee, Ill., U.S.A.), and metaxylene diisocyanate (Polysciences, Rydal, Pa., U.S.A.) were used in the conjugation procedures.

**Intracellular formation of FMDV crystals.** Primary swine-kidney-cell cultures were drained of maintenance fluid, and 0.2 ml. of the virus at a concentration of about 10^8 p.f.u./ml. was distributed over the cells. After several redistributions during incubation for 90 min. at 37°, 5 to 10 ml. of maintenance fluid (Hanks's balanced salt solution with lactalbumin hydrolysate and 2% bovine serum) was added. After 5 to 6 hr when 25 to 50% of the cells had rounded, the cultures were stored overnight at 4°. They were then used for ferritin-tagging experiments.

**Preparation of samples for electron microscopy.** Infected cultures were removed from the cold, drained of fluid, and washed once in phosphate buffer (Millonig, 1961). The cells were scraped into 2 ml. of phosphate buffer and gently centrifuged at 500 g. The pellet of cells was gently resuspended in 0.2 ml. of ferritin solution. After 90 min. incubation at room temperature, the cells were washed three times with buffer, fixed for 15 to 20 min. with 1% glutaraldehyde, rewarshed, fixed 30 min. with 2% osmium tetroxide, washed again and dehydrated through graded alcohols. Samples were embedded in epoxy (Epon 812) (Luft, 1961), sectioned with a diamond knife, and observed in an RCA-EMU-3G microscope.

**Immunodiffusion in agar gel.** Antigen was prepared from infected primary swine kidney cells by ultrasonic disruption and concentration in the ultracentrifuge. This antigen was reacted with the same ferritin-conjugates used in the electron microscopy. A rabbit-anti-guinea-pig globulin was used to test the specificity of ferritin-conjugation to γ-globulin.

**RESULTS**

A survey of many sections in repeated experiments revealed that about one of every 25 to 30 cell profiles contained a virus crystal. Crystals were found both inside the cytoplasm where they were inaccessible to the ferritin + antibody conjugates and also in intercellular spaces between intact and disrupted cells where the reaction of antigen with ferritin-antibody could take place. Some crystals were composed of complete virus particles and others were made up of empty particles. A few crystals were observed that contained mainly empty particles with a few interspersed complete particles.

Although the antibody globulin used in these experiments contained antibody to the virus infection-associated (VIA) antigen described by Cowan & Graves (1966), it was not possible to distinguish possible intracellular VIA antigen sites from those which were merely nonspecifically tagged. Plate 1a shows the positive tagging found when crystals composed of complete A1 virus particles were treated with anti-A1 ferritin-tagged globulin. The ferritin-tagged antibody attached to sites on the entire periphery of the crystal. There was a small amount of ferritin on the shreds of tissue seen in the surrounding area. The area in the lower right may have been an area of new virus formation. This attachment may have been due to the anti-VIA component or it may have been merely mechanical. Effective tagging on crystals of empty particles was also demonstrated (Pl. 1b). These particles (Graves et al. 1968) may have the same antigenic components as the whole infective virus.

When A1 virus crystals were treated with ferritin conjugated to anti-O2 globulin
(a) Crystal of FMDV (A1) complete virus particles reacted with ferritin-tagged anti-A1 globulin. The ferritin particles have attached to the outer edge of the crystal.

(b) A crystal of 'empty' FMDV (A1) virus particles reacted with ferritin-tagged anti-A1 globulin. Ferritin is seen on the periphery and in some of the interstices of the formation.

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(Facing p. 344)
(a) FMDV (A 1) virus reacted with anti-O2-globulin conjugated to ferritin. Compared to the homologous reaction, minimal amounts of ferritin are seen around the crystal. This is probably a mechanical attachment to cellular debris.

(b) FMDV (A i) virus reacted with normal guinea pig globulin conjugated to ferritin. This crystal in the intercellular space shows very little ferritin attachment except for one small area on the left side of the micrograph.

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Crystals of foot-and-mouth disease virus (Pl. 2a), the amount of nonspecific or VIA tagging was about the same as in the previous two examples. However, the main portion of the crystal had very little ferritin attached to it and there was little evidence of cross-reaction. The reaction between crystals of A\textsubscript{1} virus and untreated guinea-pig-globulin conjugate showed only minimal reaction and that may have been purely mechanical (Pl. 2b).

As a further check on the specificity demonstrated in the electron micrographs the same reagents, A\textsubscript{1} virus antigen and the several ferritin samples, were used in an immunodiffusion test in agar gel. A\textsubscript{1} antigen interacted with the homologous antibody while not with the heterologous O\textsubscript{2}-antibody. The anti-A\textsubscript{1} ferritin conjugate also reacted with rabbit-anti-guinea-pig globulin, demonstrating the specificity of the ferritin-tagging to the antivirus antibody.

DISCUSSION

The fact that specific antigen + antibody reactions occur between ferritin-tagged A\textsubscript{1} antibody and crystals of FMDV (A\textsubscript{1}) or empty particles demonstrated that the latter possess at least part of the antigenicity of the intact virus particles. Antibody to a different type of virus (O\textsubscript{2}) as well as normal\textgamma-globulin conjugated to ferritin did not react with the type A\textsubscript{1} virus crystals. Sera are now being prepared from which it should be possible to extract the antibody globulin to each of the antigens of the FMDV system (Cowan & Graves, 1966; Graves et al. 1968). Such defined fractions of specific antisera may be helpful in locating areas of virus formation and degradation by using ferritin-tagging and autoradiography.

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REFERENCES


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PLATE 3

Immunodiffusion patterns in agar gel. (a) Ferritin-tagged anti-A1 globulin in centre well and increasing twofold dilutions of rabbit-anti-guinea-pig-globulin serum in the outer wells going clockwise from the top. The ferritin is attached to the globulin fraction which acts as antigen in this test. The two bands show the reactions between both conjugated (outer) and unconjugated (inner) anti-A1 globulin.
(b) FMDV (A1) antigen in centre well and increasing twofold dilutions of anti-A1 globulin (ferritin-tagged) in outer wells proceeding clockwise from the top. The line shows the specific reaction of A1 antigen and anti-A1 globulin. (c) FMDV (A1) antigen in centre well: undiluted ferritin-tagged anti-A1 globulin at top and next clockwise well. Two lower right wells: anti-O2 ferritin-tagged globulin. Two left hand wells: normal guinea-pig globulin, ferritin-tagged. Reaction only between A1 antigen and antibody.