The Development of Colorado Tick Fever Virus Within Cells of the Haemopoietic System

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SUMMARY

Electron microscopic examination of haemopoietic liver tissue from mice infected in utero or when newborn showed inclusions of Colorado tick fever virus within erythroblasts, reticulocytes and erythrocytes. Inclusions were also seen within erythroblastoid cells undergoing mitosis. Other evidence of virus replication within erythropoietic cells was the presence of intracytoplasmic and intranuclear fibres, which have been shown to be associated with Colorado tick fever virus replication.

The findings reported here support the hypothesis that virus replication within infected erythropoietic cells occurs concurrently with differentiation of the infected cell, resulting in the presence of virions within erythrocytes.

INTRODUCTION

Colorado tick fever (CTF) virus has been demonstrated within red blood cells obtained from patients and from experimentally infected animals (Emmons & Lennette, 1966; Emmons et al. 1969; Emmons et al. 1972). Transmission of the disease by transfusion of infected donor blood has also been documented (Randall et al. 1975). The intra-erythrocytic location of the virus protects it from the circulating antibodies which begin to appear at 2 to 3 weeks after the onset of symptoms (Emmons & Lennette, 1966). Thus, peripheral blood cells from an infected man remain infectious long after the symptoms of the disease have subsided, in spite of the high concentrations of neutralizing antibodies. This carrier state persists for the lifespan of the infected erythrocytes, at the end of which time cell lysis occurs, resulting in the neutralization of the infectious virus. We have previously proposed that virus replication within infected cells of the bone marrow, concurrently with red cell differentiation of the infected stem cell, results in the presence of virus within erythrocytes. The mechanism by which CTF virus becomes established within erythrocytes, however, has not yet been demonstrated. The present study illustrates the sequence of events leading to the establishment of infectious virus particles within erythrocytes.

METHODS

Virus. The JU strain of non-neuroadapted CTF virus was used throughout these studies. The virus was passaged intraperitoneally (i.p.) in newborn mice and blood was used as the virus source. The virus was also passaged twice in BHK-21 cells to a titre of $10^8$ ID/ml and used for some of the experiments.
Animal inoculations. Newborn mice and hamsters were inoculated i.p. with 0.03 ml of inoculum. Foetuses were inoculated with 0.02 ml i.p.; pregnant mice and hamsters were anaesthetized at 2 days or at 1 week before the expected time of delivery, and the uterus exposed through an incision in the abdominal wall.

Electron microscopy. After 2, 3 and 4 days post infection (p.i.), half of the animals from each litter were sacrificed and the haemopoietic liver from each mouse was removed and individually monitored by the fluorescent antibody technique for the presence of CTF virus antigens. The antigen concentration, as determined by this procedure, was highest at the 3-day interval. Livers from those animals which showed high concentrations of virus antigens were pooled and processed for electron microscopy. The tissues were finely minced with a razor blade and single cells were obtained by stirring for 15 to 25 min in a solution containing 1% trypsin and 0.1% ethylenediaminetetraacetic acid in phosphate buffered saline (PBS), pH 7.4. The cell suspensions were filtered through several layers of gauze to remove large aggregates of tissue, and the filtrates centrifuged at 300 g for 15 min; the supernatant fluid was discarded, and the procedure repeated, using Tyrode's solution to wash the cells. The pellet of cells was resuspended in a drop of PBS, drawn into capillary tubes which were then plugged on one end with clay and centrifuged at 1000 g for 30 min. After centrifugation, the tubes were broken near both ends of the packed column of cells and fixed overnight in 3% glutaraldehyde at 4°C. The fixed column of cells was washed in 0.1 M-phosphate buffer, pH 7.2 for several hours and then fixed in 1% osmium tetroxide, rinsed in buffer, dehydrated in a graded series of ethanol concentrations, and embedded in Epon. Thin sections were stained with uranyl acetate and lead citrate.

RESULTS

Inoculation of mouse foetuses 1 week before term led to foetal death and resorption; inoculation at 2 to 3 days before term resulted in normal births, but frequently the mothers devoured their offspring (which were presumably ill although we were unable to detect any signs of illness). The livers obtained from survivors did not contain higher concentrations of virus than did tissues from mice inoculated within a few hours of birth. Hamster foetuses inoculated in utero were not affected; newborn animals showed no signs of illness over a 2-week observation period.

Fluorescent antibody monitoring of liver cells for the presence of CTF virus antigens indicated that the number of fluorescent foci increased to the highest level at 3 days p.i. and dropped off during the 4th day. Accordingly, haemopoietic liver cells from animals at 2 and 3 days p.i. were selected for electron microscopic examination. CTF virions were seen in cells from both the 2- and 3-day p.i. time periods. Even after careful selection for tissues containing the highest concentration of fluorescent foci, the number of infected cells as visualized by electron microscopy was still very low and required prolonged examination for the demonstration of virus particles.

The top layer of the column of packed cells from newborn mice at 2 and 3 days p.i., or from mice infected in utero, contained erythroblasts and hepatocytes in approximately equal numbers. Erythrocytes, reticulocytes and granulocytes were also present. The next layer of cells similarly consisted of approximately equal numbers of erythroblasts and hepatocytes, but with increased numbers of reticulocytes and erythrocytes. It was in this segment of cells that CTF virions were seen within cytoplasmic inclusions; these virions appeared similar to those seen in infected tissue culture cells (Murphy et al. 1968; Oshiro & Emmons, 1968).
Virions within haemopoietic cells

Fig. 1. An erythroblast containing CTF virus inclusions (arrows).
Fig. 2. A higher magnification of one of the inclusions shown in Fig. 1.
Fig. 3. Part of the cytoplasm of an erythroblast containing CTF virus infection mediated strands of fibres.

Fig. 4. Part of the nucleus of an erythroblast containing CTF virus infection mediated strands of fibres.

Fig. 5. A mitotic erythroblast containing a CTF virus inclusion (arrow). Inset shows higher magnification of inclusion.
Fig. 6. The nucleus being expelled from an erythroblast.
Fig. 7. A reticulocyte containing CTF virus and infection mediated strands of fibres.
Fig. 8. An erythrocyte containing CTF virus.

Fig. 1 illustrates an erythroblast containing 2 fibrillar inclusions with a few virus particles on the periphery. Occasionally, a few particles were scattered throughout the fibrillar matrix. A higher magnification of one of the inclusions is shown in Fig. 2. Fibrillar inclusions without virus particles were also noted and these presumably represent an earlier stage in the replicative process, one at which virions are not yet being produced. A few strands of intracytoplasmic (Fig. 3) and intranuclear (Fig. 4) fibres similar to those seen in infected tissue culture cells were also seen.
Virus within erythroblasts undergoing mitosis, and within late erythroblasts undergoing enucleation, was searched for, since the presence of virus, or structures associated with CTF virus infection within these cells, would provide evidence in support of the proposed hypothesis. An extensive search for the presence of virus in mitotic cells revealed three such cells. Fig. 5 illustrates one of the cells containing an inclusion (arrow) with virus particles. A higher magnification of the inclusion is illustrated within the inset.

Although uninfected cells in the process of enucleation (Fig. 6) were detected, the final transitional step in the passage of virions from nucleated cell to an enucleated one was not encountered. Extruded nuclei were seen occasionally. Fig. 7 illustrates a reticulocyte containing CTF virions and fibres. This cell was presumably an infected erythroblast which then transformed into a reticulocyte. Further differentiation of an infected reticulocyte resulted in a mature erythrocyte containing infectious virions (Fig. 8).

**DISCUSSION**

The demonstration of CTF virions within cells at the various stages of haemopoietic differentiation illustrates the mechanism by which infectious virions become established within erythrocytes. The presence of virus inclusions or 'factories' of virus production within early erythroblasts indicates that the infection had originated approximately 48 h earlier. Moreover, Schumacher & Erslev (1965) reported a period of 2 to 4 days as the time necessary for a pronormoblast to differentiate into a late erythroblast. Thus the temporal relationship implicated for one cycle of virus replication and the time estimated for a stem cell to differentiate into an early erythroblast is well within the time sequence for the mechanism suggested in our hypothesis; i.e. that virus replication within infected cells of the bone marrow, concurrently with red cell differentiation of the infected stem cell, results in the presence of virus within erythrocytes.

The presence of virus inclusions within mitotic cells provides additional evidence for the passage of virions to daughter cells. This mechanism further protects the virions from neutralizing antibodies which may have begun to appear in the peripheral blood. Although we were unable to detect virions within late erythroblasts in the process of enucleation, the normal sequence of events leading to the transformation of an erythroblast to reticulocyte (Fig. 6; Orlic et al. 1965; Skutelsky & Danon, 1967) provides a mechanism for the final step in the passage of virions into an erythrocyte. The difficulty in demonstrating virions within infected, enucleating erythroblasts is probably due to the low number of such cells and to the rapidity in which the process of nuclear extrusion takes place (Bessis & Bricka, 1952). There is, of course, the possibility that some virions may enter reticulocytes or erythrocytes after the transformation process. In view of our demonstration of virus inclusions, virions and infection-associated fibres within erythroblasts, this latter mechanism seems unlikely. Moreover, the long, intact strands of fibres seen within reticulocytes and erythrocytes were not enclosed within pinocytotic vesicles and the possibility of direct entry of such elongated structures into cells seems remote. We suggest that this unusual aspect of pathogenesis be studied in a similar manner for other virus infections which produce cell-associated viraemia.

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REFERENCES


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