The Correlation of Fatty Acid Content of Infected Cells and Virions with Newcastle Disease Virus (NDV) Virulence

(Accepted 21 August 1980)

SUMMARY

Chick embryo fibroblasts (CEF) infected with virulent strains of Newcastle disease virus (NDV) showed a dramatic increase in total unsaturated fatty acids (UFA). This increase was not seen in cells infected with the avirulent strains of NDV, Sendai virus or influenza A (PR8). The virions of the virulent strains of NDV harvested from the chorioallantoic cavity also had a higher UFA content compared to the avirulent ones. The kinetics of UFA increase in the virulent strains could be correlated with published data on the kinetics of RNA and protein synthesis inhibition, polykaryon formation and membrane permeability changes.

The envelope of paramyxoviruses consists of a lipid bilayer (the virus membrane) on the outside of which are the virus projections (spikes). The virus membrane is derived from the host cell and its lipid composition reflects the composition of the host cell membrane in all respects except for the host proteins, which are replaced by virus proteins. Moreover, the glycolipid antigenic determinants of the host membrane are preserved in the virus membrane (Apostolov & Sawa, 1976). The paramyxoviruses penetrate the host cell by fusion, are capable of haemolysis and fusion in vitro, and also produce syncytia in infected cells. These functions are properties of an integral virus envelope and are commonly referred to as fusogenic properties. There is convincing evidence that the envelopes of paramyxoviruses contain a fusion protein (F), which is involved in the fusogenic properties and which, in some cells, is cleaved from a precursor protein (F0) by a host protease (Scheid & Choppin, 1974). It has also been shown that cells infected with virulent strains of NDV have a greater capacity to cleave the F0 protein and thus promote the spread of the virus (Nagai et al., 1976). However, for the expression of the fusogenic properties by the virus envelope the lipids are essential (Hosaka, 1975). Recently, we demonstrated that the fusogenic properties of paramyxoviruses depend on the degree of saturation of fatty acids (Apostolov, 1980; Blenkharn & Apostolov, 1980). These results prompted us to undertake this study.

The Newcastle disease virus (NDV) strains, and data on their virulence, were obtained from Dr W. H. Allan, Central Veterinary Laboratory, Weybridge, Surrey. The viruses were grown in 11-day-old chick embryos, and the allantoic fluids harvested after a minimum of 48 h incubation at 35 °C. Chick embryo fibroblast (CEF) monolayers were prepared by a standard method (Apostolov & Sawa, 1976). These were distributed in tissue culture tubes, containing approx. 10^5 cells. When confluent, and after washing three times with phosphate-buffered saline, the cultures were inoculated with 128 HAU/tube with virus at a multiplicity of infection greater than 100/cell. After washing in minimal essential medium (MEM) without serum, the cultures until harvested were incubated in MEM with added bicarbonate but with no serum. At the appropriate time intervals, extraction of the total tissue culture lipids, fatty acid derivation and analysis was performed as previously described (Blenkharn & Apostolov, 1980).

In this study, in addition to the uninfected control, we have examined the UFA content of cells infected with five virulent (velogenic) NDV strains and two avirulent (lentogenic) live vaccine strains (Fig. 1). We also examined Sendai and influenza A (strain PR8) viruses. The
Fig. 1. Kinetics of UFA content of myxovirus-infected CEF. The cultures were infected with chick embryo-grown viruses at an m.o.i. greater than 100/cell. At intervals, the lipids were extracted and the percentage of total UFA determined. Data from two separate experiments are shown. The virulence data are given in Fig. 2. (Strains Finland and Paraguay are similar to strain Texas.)

results presented in Fig. 1 are from two experiments; the curves for the control and the Texas and Ulster strains were repeated under identical experimental conditions. The variation in UFA was ±4% between the two experiments. It is obvious that the virulent NDV strains (AG68V, Texas, Herts, Finland and Paraguay) had a drastic effect on the lipid metabolism of the host cell, resulting in a progressive increase in UFA within the first 15 to 20 h, continuing up to 72 h. On the other hand, the avirulent strains (Ulster and AG68L) showed a decrease in UFA content during and after this time to levels below those of the control cells. Sendai virus, in this respect, behaves like an avirulent NDV strain, as does influenza virus (PR8 strain).

The predominant fatty acids of uninfected CEF, highly purified virions and adult chick erythrocytes (RBC) were cis-9-octadecenoic, cis-9-cis-12-octadecadienoic, cis-9-icosenoic and cis-9-hexadecenoic acids, and it was these acids which showed the greatest changes throughout the kinetic studies presented in Fig. 1.

It is interesting to note that the kinetic curves for UFA increase in the virulent NDV strains correlate well with the kinetics of the increase in polykaryon formation (fusion from within), an established criterion for virulence of NDV strains (Reeve & Poste, 1971). There is also a similar correlation with the kinetics of the increase in membrane permeability of CEF after infection with the Texas strain (Katzman & Wilson, 1974). However, more important is the correlation with the progressive inhibition of RNA and protein synthesis demonstrated for the virulent strains of NDV (Lancaster & Alexander, 1975). In contrast, the avirulent strains did not produce any of these changes.

In Fig. 2 we present data on fatty acid analysis performed in duplicate of viruses harvested from the chorioallantoic cavity of chick embryos. The virions were partially purified and concentrated by differential centrifugation at 20000 g for 20 min and then further purified by zonal centrifugation with resultant purity of 40000 HAU/mg (Reeve & Alexander, 1970; Sawa, 1979). It can be seen that the virulent strains have two to four times higher ratios of UFA compared with the avirulent ones, whilst the mesogenic strains fall midway between the
two, as do the RBCs. In addition, the analysis of the UFA distribution within the phospholipid, glycolipid and neutral lipid fractions of the total virion lipids showed uniform distribution of UFA within these three fractions (data not presented). In this context it is worth noting that in vitro studies with the virions of strain Texas produced a higher fusion index than in vitro studies with the avirulent strain Ulster using chick erythrocytes (Terry & Ho-Terry, 1976). It is also interesting that the velogenic strain AG68V, the parent strain of the lentogenic AG68L obtained by a high number of egg passages (Allan & Borland, 1980), produced higher UFA in the cells as well as in the virions (Fig. 1 and 2). Apparently, the UFA composition of the virions reflects the UFA composition of the cells harvested at an early stage in the replication cycle. This finding could be an explanation for the higher liquidity of VSV-infected cells compared to the VSV virions found by Patzer et al. (1978) when examined using fluorescent depolarization techniques.

The current concept on the fluidity of cell membranes is that it depends on the overall content of UFA of the constituent lipids of the membrane (Chapman, 1975; Chapman & Quinn, 1976; Schroit & Gallily, 1979). The higher the content of UFA the higher the mobility of the lipid bilayer. Catalytic hydrogenation (saturation) of UFA in a system of artificial lipid bilayers (liposomes) was shown to lead to abolition of their mobility and reactivity (Chapman & Quinn, 1976).

In our recent work we showed that iodination of NDV and Sendai viruses under defined conditions leads to selective inhibition of haemolysis, fusion and infectivity, with no effect on haemagglutination titre or neuraminidase (Apostolov, 1980). We also demonstrated that there was a direct correlation between the degree of inhibition of haemolysis and the degree of saturation of UFA in the lipids of the virus (Blenkharn & Apostolov, 1980). On the basis of these studies, it was postulated that the fusogenic properties of paramyxoviruses depend on the mobility of the virus membrane, which in turn depends on the content of UFA. Saturation of carbon–carbon double bonds by iodine leads to an increase in melting points of membrane lipids and thus to decreased mobility, which in turn leads to loss of fusogenic capacity. In this paper we have presented data concerning the fatty acid composition of virus-infected cells; a similar correlation between UFA content and virus virulence is noted. It seems apparent that alteration of UFA content could be responsible for the appearance of toxic and virus-specific cytopathic effect. Work is in progress to demonstrate this effect with toxins and other viruses.

**Fig. 2. Percentage of UFA content of NDV virions grown in the allantoic cavity of chick embryos.** Zonal density gradient-purified virions (40,000 HAU/mg protein) were extracted and the UFA content determined by gas-liquid chromatography. Adult chick erythrocytes (RBC) were used as reference. Virulence data are given as intravenous pathogenicity index (IVPI) and mean death time (MDT). NA, Not available.
Short communications

We thank Professor A. P. Waterson for reading and commenting on the manuscript, and W. Barker for excellent technical assistance.

Departments of \(^1\)Bacteriology and \(^2\)Virology, Royal Postgraduate Medical School, London W12 0HS, U.K.

J. I. BLENKHARN\(^1\)*
K. APOSTOLOV\(^2\)

REFERENCES


(Received 27 May 1980)