Measles Virus Strain-dependent Variation in Outcome of Infection of Human Blood Mononuclear Cells

By JORMA ILONEN, REIJO SALONEN, RAYMOND MARUSYK AND AIMO SALMI*

Viral Pathogenesis Research Unit, Department of Medical Microbiology and Infectious Diseases, Faculty of Medicine, University of Alberta, Edmonton, Alberta T6G 2H7, Canada

(Accepted 22 September 1987)

SUMMARY

Eight measles virus strains including four subacute sclerosing panencephalitis (SSPE) isolates were compared on the basis of their growth characteristics in human peripheral blood mononuclear cells and effect on mitogen-stimulated lymphocyte proliferation. The Edmonston strain virus and some other strains of measles virus replicated in phytohaemagglutinin (PHA)-stimulated lymphocytes and released high titres of infectious virus into the culture medium. Inhibition of DNA synthesis was relatively low at the beginning of lymphocyte proliferation and could be detected only at high multiplicity of infection. Three to 4 days after initiation of proliferation a strong inhibition was seen also in cultures infected with low doses of virus, and was apparently related to the production of high titres of new infectious virus. Some virus strains originally isolated from SSPE patients produced only a small amount of infectious virus in lymphocytes at 37 °C but had a very strong inhibitory effect on lymphocyte proliferation leading to early cell death. The inhibitory effect was found over a wide range of virus concentrations, but was strictly dose-dependent and no increase in inhibition with low multiplicities of infection could be seen with longer culture times. The amount of interferon induced by different strains varied from 400 to 6400 international units/ml but the amount of interferon produced in the cultures did not correlate with the inhibitory effect on proliferation or with the amount of released new infectious virus. The Hallé measles strain, originally isolated from an SSPE patient, differed most from the Edmonston strain in its characteristics, and was studied in greater detail. Although only a small amount of infectious virus was produced, the Hallé strain had a very strong inhibiting effect on the proliferation of PHA-stimulated lymphocytes, and extensive syncytium formation was seen leading to cell death within 3 days.

In addition to the well known clinical infection, measles virus causes the rare disease subacute sclerosing panencephalitis (SSPE) after years of latency (Zeman, 1978). The virus may also have a role in the development of certain chronic diseases such as multiple sclerosis (ter Meulen & Stephenson, 1983) and Paget's disease (Baslé et al., 1985, 1986).

It has been speculated that measles virus with different growth characteristics may be involved in late sequelae like SSPE, but studies of strains isolated from SSPE patients have not revealed any common pattern characterizing these viruses (Wechsler & Meissner, 1982). The comparison of growth characteristics between various SSPE-derived and wild-type strains has usually been made in various tissue culture cell lines and brain cells. However, the infection of lymphoid cells may have a role in the development of diseases like SSPE through latent infection (Griffith, 1985; Fournier et al., 1985).

The inhibitory effect of measles virus infection on lymphocyte function in vivo is well known (von Pirquet, 1908, Finkel & Dent, 1973). The infection of peripheral blood mononuclear (PBM)
cells *in vivo* leads to a silent infection, but mitogenic stimulation of lymphocytes leads to virus replication and release of infectious virus. This is associated with a strong inhibition of cell proliferation and ultimately with cell death (Sullivan et al., 1975a, b; Lucas et al., 1977). Most studies on the infection of human lymphoid cells have been done using the Edmonston strain of the virus.

In this paper we report results from comparison of the infection of PBM cells with a group of measles virus strains including strains originally isolated from SSPE patients. We believe that the data obtained in this study are indicative of the presence of a biological activity marker(s) for the differentiation of measles virus strains.

PBM cells were isolated from heparinized venous blood by Ficoll-Paque™ (Pharmacia) gradient centrifugation. After three washings with phosphate-buffered saline (PBS), the cells were suspended in RPMI 1640 culture medium supplemented with 10% foetal calf serum (FCS), antibiotics and bicarbonate. Cultures were established either in microtitre plates or tissue culture tubes and incubated in a humidified 5% CO2 atmosphere for the time indicated. The cell density in the microtitre plates was 0.25 × 10⁶/ml and in tissue culture tubes 1.0 × 10⁶/ml. The phytohaemagglutinin (PHA-P, Sigma) was used at a final concentration of 10 μg/ml which was found to give maximal stimulation. [³H]Thymidine was added to microtitre plate cultures for the final 24 h and the amount of incorporated radioactivity measured by liquid scintillation spectrometry after harvesting on glass fibre filters.

Stock virus was grown in roller bottle cultures of Vero cells at 32 °C and at the time of maximal c.p.e. the supernatant was clarified and frozen in small aliquots. Virus strains were obtained from the following sources: Edmonston (American Type Culture Collection); VSC (wild-type isolate), Bray, Berg, Mantooth, Woodfolk (from Dr D. L. J. Tyrrell of this department); Hallé (from Dr T. F. Wild, INSERM, Lyon, France); Lec-KI (from Dr E. Norrby, Karolinska Institute, Stockholm, Sweden). The strains Hallé, Berg, Lec-KI and Mantooth were originally isolated from SSPE patients. All had been maintained in this and other laboratories for several passages prior to use in this study.

For plaque titration supernatants were collected from tube cultures at the indicated time, centrifuged (1000 g), and stored at −70 °C until plaque titration using standard methods on Vero cells at 37 °C.

Virus stocks diluted to the appropriate concentration of infectious virus with RPMI 1640 medium were incubated with PBM cells for 1 h at 37 °C after which the cells were washed once with PBS and the cells suspended in fresh medium supplemented with FCS and PHA when indicated.

Supernatants to be tested for interferon were collected after 48 h culture time and stored at −70 °C until tested. A standard c.p.e. inhibition assay was used for measuring the amount of interferon-alpha (IFN-α). In short, 7 × 10⁴ bovine kidney cells (MDBK) in 100 μl of MEM supplemented with 2% FCS were added to each well of 96-well microtitre plates. Samples with interferon were immediately added to the cells in serial two-fold dilutions and incubated overnight at 37 °C. The cells were infected with vesicular stomatitis virus (5 p.f.u./cell) and c.p.e. was examined after overnight incubation at 37 °C. The interferon titre was determined based on a 50% reduction in c.p.e. An international reference IFN-α preparation (Professor K. Cantell, National Public Health Institute, Helsinki, Finland) was included in each assay to allow the calculation of international units (IU). This test system measures mainly IFN-α as the MDBK cell line is very sensitive to this interferon (Gresser et al., 1974) and insensitive to IFN-γ (Lebon et al., 1979).

Surface labelling for measles antigen was done by incubating 2 × 10⁵ cells with 1:40 diluted monoclonal anti-haemagglutinin (H) antibody in an ice bath for 30 min. Cells were then washed twice with PBS and the procedure was repeated with a 1:40 diluted fluorescein isothiocyanate-conjugated anti-mouse antibody (Kirkegaard & Perry Laboratories, Gaithersburg, Md, U.S.A.). After further washings, cells were suspended in a glycerol–PBS mixture (1:1), a drop was placed on a microscope slide, covered, and examined with a fluorescence microscope.

Wide variation was found in the amount of infectious virus released from PHA-stimulated mononuclear cells infected by different virus strains (Table 1). The highest levels were found in
cultures infected by the Edmonston strain whereas no virus or very low levels of virus were detected in the Hallé strain-infected cultures. An apparent inverse correlation between infectious virus production and blast transformation was found, the Hallé strain being the most inhibitory.

The kinetic study of the proliferation of cells infected at various multiplicities showed clear differences between the strains tested (Fig. 1). When infected by the strains Edmonston, VSC, Bray or Woodfolk, an early inhibition of proliferation after 2 days could be seen only at a high m.o.i. and was relatively low. The relative inhibition increased with time and at day 4 a strong inhibition could be seen over a wide range of initial infective doses. In the other group of strains (Mantooth, Lec-KI, Berg and Hallé), the inhibition was strong from the beginning and was seen over several dilutions. The actual strength of the inhibition usually remained strictly related to the initial dose throughout the culture time.

There was also a wide variation in the amount of IFN-α produced in cultures infected with the different measles strains; values between 400 IU/ml and 6400 IU/ml were found. The amount of interferon in cultures did not correlate with either the proliferation inhibition, the release of new infectious virus or the strain of infecting virus as both the Hallé and Edmonston strains produced similar interferon levels.

The immunofluorescence study of infection revealed that in the PHA-stimulated Edmonston strain-infected cells surface antigen appeared 2 days post-infection (p.i.) and by 3 to 4 days p.i. the majority of the cells were antigen-positive. Although antigen-positive cells were often clumped, separate cells could usually be clearly recognized. At 2 days p.i. with the Hallé strain, few individual cells were surface antigen-positive, but several syncytia were present with a unified brightly fluorescent membrane. This was followed by rapid cell death with few viable cells left at 3 days p.i.

The comparison of measles virus strains was done at 37 °C in order to mimic the temperature of natural infection. The growth characteristics of the Hallé strain in PBM cells changed when cultures were incubated at 32 °C instead of 37 °C. In a comparison of PHA-stimulated Edmonston and Hallé strain-infected cultures (4 p.f.u./cell), higher titres of the Hallé strain were produced when cultures were incubated at 32 °C (7 × 10⁴ and 1 × 10⁶ p.f.u./ml in 3 and 5 days old cultures of Hallé-infected compared to 2 × 10⁴ and 3 × 10⁴ p.f.u./ml in Edmonston strain-infected cultures). In parallel cultures incubated at 37 °C, the reverse was seen (9 × 10² and 0 p.f.u./ml at 3 and 5 days for Hallé strain-infected and 2 × 10⁴ and 4 × 10³ for Edmonston strain).

### Table 1. Release of infectious virus* from PBM cells infected with 4 p.f.u./cell of different strains of measles virus and cultured at 37 °C

<table>
<thead>
<tr>
<th>Strain</th>
<th>PHA</th>
<th>0</th>
<th>1</th>
<th>3</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edmonston</td>
<td>-</td>
<td>8 × 10³</td>
<td>6 × 10¹</td>
<td>1 × 10²</td>
<td>3 × 10²</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>ND</td>
<td>6 × 10¹</td>
<td>6 × 10⁴</td>
<td>3 × 10⁵</td>
</tr>
<tr>
<td>VSC</td>
<td>-</td>
<td>2 × 10⁴</td>
<td>1 × 10²</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>ND</td>
<td>1 × 10²</td>
<td>4 × 10³</td>
<td>1 × 10⁴</td>
</tr>
<tr>
<td>Bray</td>
<td>-</td>
<td>6 × 10⁴</td>
<td>8 × 10³</td>
<td>5 × 10¹</td>
<td>1 × 10¹</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>ND</td>
<td>9 × 10³</td>
<td>2 × 10⁵</td>
<td>2 × 10⁵</td>
</tr>
<tr>
<td>Woodfolk</td>
<td>-</td>
<td>4 × 10³</td>
<td>9 × 10¹</td>
<td>4 × 10¹</td>
<td>7 × 10¹</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>ND</td>
<td>2 × 10¹</td>
<td>6 × 10²</td>
<td>3 × 10⁴</td>
</tr>
<tr>
<td>Mantooth</td>
<td>-</td>
<td>5 × 10⁴</td>
<td>6 × 10¹</td>
<td>2 × 10¹</td>
<td>5 × 10¹</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>ND</td>
<td>1 × 10²</td>
<td>4 × 10⁴</td>
<td>3 × 10⁴</td>
</tr>
<tr>
<td>Lec-KI</td>
<td>-</td>
<td>6 × 10³</td>
<td>4 × 10²</td>
<td>1 × 10²</td>
<td>4 × 10¹</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>ND</td>
<td>2 × 10⁴</td>
<td>2 × 10³</td>
<td>2 × 10²</td>
</tr>
<tr>
<td>Berg</td>
<td>-</td>
<td>2 × 10⁴</td>
<td>8 × 10²</td>
<td>2 × 10¹</td>
<td>2 × 10¹</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>ND</td>
<td>6 × 10²</td>
<td>1 × 10⁴</td>
<td>8 × 10³</td>
</tr>
<tr>
<td>Hallé</td>
<td>-</td>
<td>7 × 10³</td>
<td>3 × 10²</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>ND</td>
<td>3 × 10²</td>
<td>1 × 10¹</td>
<td>0</td>
</tr>
</tbody>
</table>

* Titres given in p.f.u./ml.
† ND, Not determined.
The inhibition of PHA-induced lymphocyte proliferation was also similar with both strains when cultures were incubated at 32 °C instead of 37 °C.

The low amount of infectious virus produced at 37 °C cannot be explained by rapid cell death and the loss of virus-producing cells, as similar results were obtained when cells were infected at a low multiplicity. At an m.o.i. of 0.1, the virus released from the few originally infected cells would have the opportunity to undergo several cycles of replication in proliferating cells. The growth of the Hallé strain to higher titres in Vero cells at 32 °C than at 37 °C has been reported earlier and is associated with the temperature-dependent production of defective interfering particles (Tsang et al., 1981).

The Hallé and Berg measles strains quickly inhibit cell proliferation even at low m.o.i., a condition under which only a small number of cells could be initially infected. However, a low m.o.i. with other strains induced strong inhibition at 3 to 4 days p.i. At that time, the inhibition mediated by a low concentration of the Edmonston strain virus was greater than that caused by
an equivalent dose of the Hallé strain virus. When cultures infected with various doses of the Edmonston strain virus were incubated after infection in RPMI medium supplemented with human serum containing neutralizing antibodies in place of FCS (data not shown), no late inhibition was seen. Only high doses of virus had an inhibitory effect throughout the incubation period. The late infection was probably related to the production of new infectious virus and infection of new cells.

Volckaert-Vervliet et al. (1978) found an inverse correlation between interferon induction and c.p.e. and virus production when the Namalwa lymphoblastoid cell line was infected with different measles virus strains. In this study, there was no clear correlation between interferon induction capacity and either virus production or inhibition of proliferation, although the variability between various strains in interferon induction could be confirmed. The interferon in the system may have an obvious, though controversial effect: the antiviral effect should support lymphocyte proliferation but on the other hand interferon also has an antiproliferative effect. The situation is further complicated by the existence of several lymphoid subpopulations and monocytes in PBM cells.

The strains with the strongest inhibitory effect on cellular proliferation were those originally isolated from SSPE patients. Whether this is truly a property of the original isolates is unknown as the isolates studied have all undergone long periods of laboratory propagation. However, the finding of differences in the characteristics of lymphocyte infection suggests that the effect of measles infection on immune regulation may also be dependent on the particular strain involved.

Measles virus is generally considered to be relatively stable antigenically with only one major neutralization type thus far detected in different parts of the world. It has been reported that minor variations can be detected with monoclonal antibodies (Sheshberadaran & Norrby, 1986). Differences in biological activity have also been observed among measles virus strains. Certain strains can induce different types of c.p.e. (Carrigan, 1985) and other strains have a low haemagglutinating capacity (Marusyk & Tyrrell, 1984). This work adds another variable, the capacity to infect and suppress functions of lymphoid cells, to the known biological variations of measles virus. The observed variation is not unexpected since all RNA viruses show a great number of minor variations in their primary gene sequences (Holland et al., 1982). Although these differences may seem trivial from the point of view of virus structure or major antigenic determinants, such small changes can have a significant effect on virus pathogenesis or disease epidemics (Hughes et al., 1986).

It is tempting to speculate that the establishment of a chronic infection in lymphoid cells in SSPE (Fournier et al., 1985) depends on the nature of the initial virus–lymphocyte interaction. Minor variations in the invading virus isolates or a rapid emergence of variants during the first cycle(s) of infection in vivo may well lead to chronic sequelae of measles infection.

We wish to thank Mrs Linda Chui, Ms Barbara Folkins and Ms Seema Dosaj for excellent technical help. The study was supported by Alberta Heritage Foundation for Medical Research and by the Medical Research Council of the Finnish Academy.

REFERENCES


(Received 26 May 1987)