Measles Virus Persistence in Human Lymphocytes: A Role for Virus-induced Interferon

By STEVEN JACOBSON* AND HENRY F. MCFARLAND

Neuroimmunology Branch, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, Maryland 20205, U.S.A.

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SUMMARY

Because of the association of measles virus with persistent infections such as subacute sclerosing panencephalitis, and its possible involvement in multiple sclerosis, we have investigated the capacity of this virus to establish chronic infections in human peripheral blood lymphocytes (PBLs). We have demonstrated that a latent, persistent infection of human PBLs with measles virus results in low levels of infectious virus production in which large amounts of virus-induced interferon could be detected. Further, treatment of these silently infected cells with an anti-human leukocyte interferon serum results in a productive measles virus infection. The mechanism by which the anti-interferon serum shifts the virus–cell interaction from persistence to productive infection is discussed.

INTRODUCTION

The established causal relationship of measles virus infection to subacute sclerosing panencephalitis (Horta-Barbosa et al., 1971) and the suggestion that measles virus may also be a causal factor in multiple sclerosis (Hasse et al., 1981) has prompted interest in the capacity of this agent to establish persistent infections. Measles virus persistence has been examined in numerous animal models, and both virus and host factors have been implicated (Rammohan et al., 1980, 1981). Less is known about mechanisms of persistence in human cells. This report attempts further to define the ability of measles virus to persist in human peripheral blood lymphocytes.

Measles virus has been previously shown to persistently infect human lymphocytes (Sullivan et al., 1975). Infection of normal, resting lymphocytes resulted in a 'silent' infection in which small amounts of infectious virus were produced and no significant expression of virus antigens on cell membranes occurred (Lucas et al., 1978a; Joseph et al., 1975). The apparent block of measles virus replication in these 'silently' infected human lymphocytes was overcome by the addition of mitogen (Lucas et al., 1978b).

There is a variety of mechanisms by which a virus can persist for long periods of time. The role of interferon as a regulatory substance is of intense interest today (Epstein, 1977). With more advanced and refined interferon purification techniques, specific antisera to interferons have become available and have greatly added to our ability to elucidate those mechanisms in which interferons play a role. In the present study we have analysed the effects of an anti-human leukocyte interferon serum on 'silent', measles virus-infected human peripheral blood lymphocytes and have shown that this leads to a productive measles virus infection.

METHODS

Cells. Human peripheral blood lymphocytes (PBL) from healthy normal volunteers were isolated by centrifugation over Ficoll–hypaque gradients (LSM, Litton Bionetics Inc., Kensington, Md., U.S.A.). Lymphocytes were infected with the Edmonston strain of measles virus at a multiplicity of infection (m.o.i.) of 1.0 at 37 °C for 1.5 h in a humidified CO₂ incubator. The lymphocytes were then placed in 15 ml culture tubes (Falcon plastics; cat. no. 3033) at a concentration of 2 × 10⁶ cells/ml in RPMI-1640 medium (Gibco), supplemented with
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10% foetal calf serum (FCS), glutamine and antibiotics. Pokeweed mitogen (PWM; Gibco, lot no. 15N 8022 cat. no. 670-5360) was used at a final dilution of 1:50. Measles virus-infected PBLs were either treated with a potent anti-human leukocyte interferon preparation (NIH reference reagent no. G-026-502-568, which has an interferon neutralizing titre of 750,000 per 0.5 ml against 8 to 10 international units (I.U.) of interferon and contains low levels of antibodies to human fibroblast interferon) or control antisera (NIH reference reagent no. G-027-501-568) at final concentrations of 1:500 and 1:100, respectively. The characteristics of these sera are outlined in the NIH/NIAID research reference reagent notes 22 and 23. Cultured lymphocytes were incubated for various times and the supernatants harvested and frozen at −70°C until assayed for interferon and infectious measles virus. The cell pellets were resuspended in 0.2 ml of phosphate-buffered saline, without calcium and magnesium (PBS−), and studied by immunofluorescence.

**Immunofluorescence.** For detection of cytoplasm-associated measles virus antigens, 100 μl of the resuspended cell pellets were spun in a centrifuge for 5 min at 300 g, acetone-fixed and incubated for 30 min at room temperature with a 1:20 dilution of a fluorescein-conjugated IgG fraction of an SSPE serum (a generous gift of K. Rammohan). For detection of membrane-associated measles virus antigens, 100 μl of the resuspended pellet was incubated for 30 min at room temperature with 35 μl of a 1:20 dilution of the fluoresceinated SSPE conjugate, washed and the percentage of positive cells determined.

**Vero monolayer plaque assay.** Infectious measles virus was measured by incubating 0.25 ml of serial 10-fold dilutions from supernatants of cultured PBLs on confluent monolayers of Vero cells. After 1.5 h, an overlay containing equal volumes of 1.0% agarose ME (Seakem, Me., U.S.A.) and 2X Eagle’s minimum essential medium (MEM; Gibco) supplemented with 10% foetal calf serum (FCS) was added. Five days later, the cultures were counterstained with 0.02% neutral red to allow counting of plaques. Data are represented as plaque-forming units per ml (p.f.u./ml).

**Interferon assay.** Supernatants from measles virus-infected PBLs were assayed for interferon activity by measuring the inhibition of encephalomyocarditis virus (EMC) cytopathic effect (c.p.e.) on fibroblast cells trisomic for chromosome 21 (GM 2504 cells). (Both the EMC virus and GM 2504 cells were kind gifts of O. Preble.) Serial two-fold dilutions of cultured PBL supernatants were added to 96-well plates (Costar, Cambridge, Ma., U.S.A.; cat. no. 3596) in a final volume of 50 μl in MEM + 10% FCS. Fifty μl of GM 2504 cells was then added, to a concentration of 1.5 × 10⁴ cells/well. After an overnight incubation at 37°C, 50 μl of EMC virus was added to a concentration of 3 × 10⁴ p.f.u./well. Plates were incubated for an additional 24 h, at which time c.p.e. was noted. To aid counting, the wells were counterstained with 0.1% crystal violet in 20% ethanol.

Each assay was standardized by the inclusion of a control, human leukocyte interferon preparation (NIH reference reagent no. G-023-901-527) of 100 I.U./ml. This assay is much more sensitive than that used to define I.U. [in particular by the use of EMC virus rather than the more conventional vesicular stomatitis virus (Berger et al., 1980)] and 1 laboratory unit/ml of interferon as determined in this assay equals 0.05 I.U./ml.

**Lymphocyte proliferation determination.** Infected and uninfected PBLs were incubated at a concentration of 4 × 10⁵ cells/well at 37°C in 96-well microtitre plates and were either left untreated, stimulated with PWM or treated with anti-leukocyte interferon. On the days indicated, cells were pulsed with 1 μCi of [³H]thymidine (New England Nuclear) for 4 h and harvested on a multiple automated sample harvester (MASH II; Microbiological Associates, Walkersville, Md., U.S.A.). Data are presented as c/min of treated measles virus-infected or uninfected cultures minus c/min from untreated cultures (i.e. difference in c/min).

**Infectious centre assay.** This assay was similar to the virus-producing assay described by Nowakowski et al. (1973). Lymphocytes cultured under different conditions were resuspended in 0.2 ml of PBS− and the number of viable cells determined by trypan blue exclusion and counting in a haemocytometer. Serial 10-fold dilutions of cells were made in an overlay containing equal volumes of 1.0% agarose ME and 2X Eagle’s MEM supplemented with 10% FCS plus 1.0% human AB serum. The latter had a measles virus radioimmune assay of 1:1000. Each dilution was plated in 0.5 ml duplicates, on to confluent monolayers of Vero cells in 60 mm² tissue culture dishes (Falcon plastics; cat. no. 3002). This thin layer of agarose/cell mixture hardened quickly and an additional 5 ml agarose/MEM overlay was added. The plates were incubated for 4 days in a humidified CO₂ incubator and a neutral red counterstain was added to facilitate enumeration of plaques. The number of plaques multiplied by 2, then by the dilution factor equals the number of infectious centres per culture.

**RESULTS**

**Effect of anti-human leukocyte interferon on the production of measles virus in infected peripheral blood lymphocytes**

Infection of resting PBLs with the Edmonton strain of measles virus resulted in the production of low levels of infectious virus. Maximum virus production was reached at 2 days post-infection and remained constant for the entire observation period (8 days). Approximately 400 p.f.u. were detected in the supernatants from 2 × 10⁶ infected PBLs (Fig. 1). Fluorescence
microscopy showed membrane-associated measles virus antigens in less than 10% of infected cells. In contrast, cytoplasmic virus antigens were found in 50 to 60% of these cells.

Stimulation of measles virus-infected lymphocytes by PWM resulted in a constant increase in measles virus production (Fig. 1). Approximately $1 \times 10^4$ to $4 \times 10^4$ p.f.u. were produced by $2 \times 10^6$ PBLs. This represented a 100-fold increase over that produced by non-stimulated, 'silently' infected cells. Moreover, 80 to 90% of infected lymphocytes stimulated by PWM expressed both surface and cytoplasmic measles virus antigens.

When an anti-human leukocyte interferon preparation was added to measles virus-infected, unstimulated PBLs, there was an enhanced production of infectious measles virus which peaked 6 to 8 days post-infection at approximately $1 \times 10^4$ p.f.u./$2 \times 10^6$ PBLs (Fig. 1). Most of the cells (80 to 90%) contained both cytoplasmic and membrane surface measles virus antigens. A control serum not containing antibody against interferon had no such stimulatory effect. While the addition of the anti-interferon serum to infected PBLs appeared to mimic the effects of PWM, production of infectious virus in the mitogen-stimulated lymphocytes was, in most experiments, somewhat higher than that seen with the anti-interferon serum treatment. The addition of the anti-interferon serum, however, always produced significantly more infectious measles virus than that observed in unstimulated, resting lymphocytes (Fig. 1).

**Ability of anti-human leukocyte interferon to act as a mitogen**

The possibility that the increased virus production in 'silently' infected PBLs was due to a mitogenic effect of the anti-interferon preparations was examined next. Infected and uninfected lymphocytes were treated with either the anti-interferon serum, a control serum not containing anti-interferon antibody or PWM. The lymphoproliferative responses of these treated PBLs are shown in Fig. 2. In contrast to the response obtained when PBLs were incubated with PWM, no increased proliferation of either infected or uninfected lymphocytes occurred when these cells
were cultured with either the control or the anti-interferon serum. The response to PWM was reduced in stimulated measles virus-infected lymphocytes as had been reported previously (Lucas et al., 1977). We conclude from these data that the ability of anti-human leukocyte interferon to induce high levels of infectious measles virus from resting, virus-infected lymphocytes (Fig. 1) is not mediated by its possible mitogenic properties.

**Effect of anti-human leukocyte interferon on production of measles virus-induced interferon in infected peripheral blood lymphocytes**

Since a mitogenic effect of the anti-interferon preparations could not explain the increased virus production in 'silently' infected PBLs (Fig. 2), we examined the stimulation of interferon by measles virus in resting lymphocytes. The induction of interferon by measles virus could account for both the latent infection in resting lymphocytes and the ability of the anti-interferon serum to increase measles virus production in these cells.

Unstimulated, virus-infected lymphocytes produced high levels of interferon by day 3 post-infection; these remained constant throughout the observation period (Fig. 3). Interferon could not be demonstrated in these same cultures after the addition of the anti-interferon serum. In contrast, interferon levels were not significantly reduced in cultures receiving the control serum without anti-interferon antibody. The interferon produced by these unstimulated, measles virus-infected PBLs was acid-stable and could be inactivated by an anti-leukocyte interferon serum, allowing it to be characterized as a human interferon alpha (HuIFN-α). The data in Fig. 3, coupled with the anti-interferon's ability to increase infectious measles virus production in 'silently' infected lymphocytes (Fig. 1), strongly argue that interferon plays a role in measles virus persistence in human peripheral blood lymphocytes.
Measles virus persistence in human lymphocytes

Table 1. Production of measles virus infectious centres in human PBLs

<table>
<thead>
<tr>
<th>Measles virus-infected PBLs*</th>
<th>Infectious centres per culture</th>
<th>Plaque-forming units/culture†</th>
<th>P.f.u. per infectious centre‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ PWM§</td>
<td>$1.4 \times 10^5$</td>
<td>$1.8 \times 10^5$</td>
<td>1.30</td>
</tr>
<tr>
<td>- PWM</td>
<td>$8.0 \times 10^3$</td>
<td>$6.0 \times 10^3$</td>
<td>0.75</td>
</tr>
<tr>
<td>- PWM + anti-IFN</td>
<td></td>
<td></td>
<td>$3.2 \times 10^4$</td>
</tr>
</tbody>
</table>

* All cultures (2 x 10^6 cells) incubated for 6 days.
† Supernatant fluids from cultured cells assayed for plaque forming units.
‡ Comparison of means of 5 experiments was not statistically significant in Student’s two-tailed t-test.
§ PWM used at a final concentration of 1:50.
|| Anti-human leukocyte interferon serum added at a final concentration of 1:500.

It is interesting to note that, when PWM was used to stimulate measles virus-infected cells to produce high levels of infectious virus (Fig. 1), a relatively high level of interferon was produced, although it was less than in unstimulated, resting lymphocytes (Fig. 3). Interferon levels were similar in both infected and uninfected cells following PWM stimulation and was acid-sensitive, indicating that immune-type interferon-γ was produced. Unstimulated, uninfected lymphocytes produced no interferon (data not shown). These data suggest that the mechanisms involved in the increased measles virus production of infected human lymphocytes by PWM treatment may not be mediated by interferon and may differ from the mechanism by which anti-leukocyte interferon operates.

Examination of mechanisms responsible for increased measles virus production following anti-human leukocyte interferon serum treatment

The addition of an anti-interferon serum to unstimulated, measles virus-infected PBLs completely inhibited the production of measles virus-induced interferon (Fig. 3) with an enhancement in measles virus production (Fig. 1). We wished to determine whether the increased yield of measles virus associated with the anti-interferon serum treatment was due to an increased efficiency of virus production in cells already infected or was due to a facilitated spread of virus to previously uninfected lymphocytes. The latter mechanism would be indicated by a constant yield of infectious virus per infected cell together with an increase in the number of infected cells.

An infectious centre assay was performed using measles virus-infected PBLs cultured with and without PWM and on infected, unstimulated lymphocytes treated with an anti-interferon serum. A representative experiment is shown in Table 1. The number of infectious centres per culture increased in cultures that were treated with either the anti-interferon serum or mitogen, compared to unstimulated, untreated cells. This coincided with an increase in the amount of infectious virus (p.f.u./ml) that could be detected in the supernatants from these same cultures (Fig. 1, Table 1). The amount of infectious virus per infectious centre, however, remained constant.

DISCUSSION

The ability of measles virus to persist in unstimulated human PBLs has been described in numerous reports (Joseph et al., 1975; Sullivan et al., 1975; Lucas et al., 1978b). In all instances, stimulation of these cells by plant mitogens (phytohaemagglutinin, concanavalin A, PWM) or allogeneic lymphocytes resulted in productive measles virus infection (Sullivan et al., 1975). The mechanisms by which these agents operate remain unknown. In the present study, we have demonstrated that a 'silent', latent infection of human PBLs with measles virus stimulates the production of large amounts of interferon. Measles virus defective-interfering particles could not be detected in these cultures (data not shown). Further, treatment of these 'silently' infected cells with an anti-interferon serum results in a productive measles virus infection.

Only one report (Lucas et al., 1978b) has measured the production of interferon in lymphocytes infected with measles virus with and without mitogen stimulation (phytohaemagg-
lutinin). In this study, only small amounts of interferon were detected in either infected or uninfected mitogen-stimulated cultures. Our data indicate that unstimulated, measles virus-infected PBLs produced high levels of interferon (Fig. 3). This discrepancy may be due to differences in our assays for interferon or the strains of measles virus used. Numerous reports have indicated that animal viruses can induce interferon in unstimulated human lymphocytes and human lymphoblastoid cell lines (for review, see Epstein, 1977). Mumps virus (another paramyxovirus) has been shown recently to induce interferon-α cultured human PBLs (Kato & Minagawa, 1981). Similarly, measles virus has been shown to induce interferon in lymphoblastoid cells (Volckaert-Vervliet & Billiau, 1977; Volckaert-Vervliet et al., 1978). These latter studies had shown that the induction of interferon by measles virus was strain-dependent.

The Edmonston strain of measles that we used (Bellini et al., 1979) induced high levels of interferon in unstimulated, resting lymphocytes. Moreover, a non-mitogenic, sheep anti-human leukocyte interferon serum completely neutralized the virus-induced interferon and increased production of infectious measles virus. Similar effects were also obtained with anti-human leukocyte interferon preparations obtained from rabbit (a gift from A. Neighbour) or goat (a gift from W. Wallen). We conclude that the ability of measles virus to persist in human PBLs is associated with the production of virus-induced interferon.

Since the anti-interferon serum augmented the production of measles virus in silently infected human PBLs, experiments were performed to examine the mechanism(s) by which this may occur. The data in Table 1 indicate that the number of infectious centres (a measure of the number of cells capable of producing infectious virus) increased in cultures containing either the anti-interferon serum or PWM as compared to unstimulated, measles virus-infected cells. There was a concomitant increase in the amount of infectious virus produced in treated cultures compared to untreated lymphocytes. Thus, the amount of infectious virus per infectious centre remained relatively constant (Table 1). These data would indicate that the effect of the anti-interferon serum on the increased production of measles virus was not due to an increase in the efficiency of a few cells to produce virus but rather by its ability to neutralize virus-induced interferon (Fig. 3), thus allowing non-infected cells to become infected. Although only a small number of infected, unstimulated PBLs had measles virus surface antigens and could produce virus in an infectious centre assay, fluorescent antibody studies indicated that a large number of these cells contain cytoplasmic measles virus antigen. It is possible, therefore, that the anti-interferon serum may induce these non-producing, infected cells to produce virus. Either mechanism would be compatible with the observed data.

It is interesting to speculate on the mechanism of virus-induced interferon on suppression of infectious measles virus. It has been shown that unstimulated, measles virus-infected PBLs synthesized 20- to 30-fold less virus protein than did PWM-stimulated lymphocytes (W. J. Bellini, G. D. Silver, D. E. McFarlin & H. F. McFarland, unpublished results). In addition, the haemagglutinin protein (HA) of measles virus was found to be incompletely glycosylated in these 'silently' infected cells. Measles virus-induced interferon could affect the pathway involved in glycosylation of measles HA so as to result in its incomplete function when in the plasma membrane, and hence reduce infectious virus yields. It has been shown that interferon can interfere with the glycosylation of vesicular stomatitis virus G protein (Maheshwari et al., 1980). In our system, the ability of an anti-interferon serum to shift a measles virus infection of human lymphocytes from a non-productive to a productive infection (Fig. 1) may occur by restoring the ability to glycosylate the HA protein fully, with its subsequent proper insertion in the plasma membrane. Recent evidence has indicated that the limited growth of Newcastle disease virus (NDV) in L cells was a result of interferon being produced during the course of the infection and inhibiting the progress of virus protein synthesis, leading to a reduction in virus yield (Nagai et al., 1981). In the presence of anti-mouse interferon antibody, the synthesis of NDV proteins progressed normally with the production of large amounts of infectious progeny (Nagai et al., 1981). Experiments in progress indicate that treatment of unstimulated, measles virus-infected lymphocytes with an anti-interferon serum may indeed cause this shift from a partially glycosylated to a fully glycosylated HA protein (W. J. Bellini, personal communication). The synthesis of all other measles virus proteins appears to be normal.
Measles virus persistence in human lymphocytes

Understanding of the mechanisms involved in measles virus persistence in human PBLs is of particular interest since this virus has been suggested to play a role in the pathogenesis of multiple sclerosis (Hasse et al., 1981). The impaired interferon responses demonstrated in lymphocytes from MS patients (Neighbour & Bloom, 1979; Neighbour et al., 1981), along with the findings in the present study, supports the idea that the nature of measles virus infections differs between these patients and normal individuals.

REFERENCES


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