Induction of Marek's Disease Virus Antigens by IdUrd in a Chicken Lymphoblastoid Cell Line

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SUMMARY

Marek's disease virus (MDV) antigens, as detected by immunofluorescence, were induced in a lymphoblastoid cell line, MSB-1, in the presence of IdUrd. When treated with 20 μg/ml of IdUrd there was no increase in the number of cells producing virus particles. If IdUrd was removed, an increase in virus production followed. Activation of the MDV genome appeared to require incorporation of IdUrd into cellular DNA and occurred during the first 12 h of culture. Expression of the activated genome required de novo protein synthesis and occurred during the next 12 h. The MDV genome in high producer MSB-1 cells could be activated with low concentrations of IdUrd, whereas low producer MSB-1 cells could not be activated with IdUrd to any great extent.

INTRODUCTION

Marek's disease is a lymphoproliferative neoplastic disease of chickens caused by a herpesvirus. Because herpesviruses have been associated with cancers of animals and man, the characteristics of virus-host interactions should be more precisely defined.

Tumours of Marek's disease (MD) and a lymphoblastoid cell line derived from such a tumour contain copies of the Marek's disease virus (MDV) genome (Nazerian & Lee, 1974). Whereas MD tumours are normally free of MDV antigens, the lymphoblastoid cell line MSB-1 produces virus antigens and virus particles in 1 to 2% of the cell population (Akiyama & Kato, 1974). These characteristics are similar to those in tumours and cell lines of Burkitt's lymphoma and other Epstein-Barr virus (EBV)-associated diseases (Klein, 1972).

Human lymphoblastoid cell lines carrying the EBV genome have been activated by 5-iodo-2-deoxyuridine (IdUrd) to produce EBV related antigens (Gergley, Klein & Ernberg, 1971a; Sugawara, Mizumo & Osato, 1973; Hampar et al. 1974; Long, Derge & Hampar, 1974). Concentrations of IdUrd greater than 20 μg/ml led to an accumulation of early antigens (EA) in producer and nonproducer cell lines. However, because only producer lines were capable of synthesizing virus capsid antigens (VCA) after removal of IdUrd, activation of the genome did not necessarily result in completion of the virus lytic cycle (Hampar et al. 1974). In addition, IdUrd activation of EBV was cell cycle dependent and occurred during replication of the resident virus genome (Hampar et al. 1973).

Data presented in this paper show that the MDV genome carried in MSB-1 cells can be activated to produce MDV-associated antigens when treated with IdUrd. Because MSB-1 cells, when treated with IdUrd, had similar properties to the EBV cell lines, the induced

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antigens were originally considered to be MDV-related early antigens (Nazerian, 1975). However, the absence of an antiserum for a specific class of MDV antigens has prevented a conclusive identification. In addition, changes in the levels of spontaneous activation of MDV in MSB-1 cells were correlated with a change in susceptibility to activation by IdUrd.

**METHODS**

**Cells and virus.** The lymphoblastoid cell line MSB-1 was provided by Dr S. Kato of Osaka University, Japan. The cell line was grown at 41°C in a 5% humidified atmosphere. MSB-1 cells were seeded in Falcon plastic Petri dishes at an initial concentration of 5 x 10^5 cells/ml. RPMI-1640 medium was obtained from Flow Laboratories and supplemented with 10% bovine foetal calf serum (BFS) and penicillin-streptomycin. Subcultures were made every 2 to 3 days. The number of viable cells was determined by trypan blue exclusion.

**Reagents.** Aminopterin was obtained from ICS Pharmaceuticals, Inc., thymidine from Calbiochem; IdUrd and cytosine arabinoside (ara-C) from Sigma Chemical Co., and hypoxanthine and cycloheximide from Aldrich Chemical Co., Inc. Concentrated stock solutions were prepared and stored at 4°C.

**Antiserum.** The anti-MD chicken serum used in this study was provided by Dr R. L. Witter. It was prepared in line 7 chickens inoculated intraperitoneally with MDV (GA strain) infected duck embryo fibroblasts.

**Activation of MDV antigens with IdUrd.** MSB-1 cells were subcultured as usual, and various concentrations of IdUrd were added to the medium and maintained for 24 to 48 h. With some cultures, cells were pelleted, washed with phosphate-buffered saline (PBS) and resuspended in normal medium for an additional 48 h. At the end of the specified time, cells were harvested and prepared for immunofluorescence (IF) or electron microscopy.

**Immunofluorescence test.** Light smears of MSB-1 cells were prepared on glass slides, air dried, and fixed in acetone at -20°C for 24 h. Slides were removed from acetone, dried under forced air and washed in PBS. The cells were incubated in a 1:40 dilution of anti-MD chicken serum for 30 min, washed for an additional 30 min, and further incubated for 20 min in a 1:20 dilution of fluoresceinisothiocyanate-conjugated anti-chicken globulin, obtained from Roboz Surgical Instruments Co., Inc. Concentrated stock solutions were prepared and stored at 4°C.

**Electron microscopy.** MSB-1 control cultures and MSB-1 cultures treated with IdUrd were pelleted and fixed in 1% osmium tetroxide for 90 min. Pellets were washed in a salt buffer, and then dehydrated in graded series of ethyl alcohol and embedded in Epon 812. Thin sections were prepared with an MT-2 Porter-Blum ultramicrotome, stained with uranyl acetate and lead citrate, and examined in an Elmiskop 1A Siemens electron microscope.

**RESULTS**

**Spontaneous production of MDV-specific antigens in MSB-1 cells**

In these experiments, the doubling time for MSB-1 cells was 10 to 14 h; and after 48 h in culture, 85 to 90% of the cells were viable. These cultures spontaneously produced MDV antigens as detected by IF and are called producers. Some cultures produced virus antigens at a low level (less than 1%) and were called low producers, whereas other cultures, with
Marek’s disease antigen induction by IdUrd

Fig. 1. Growth curve of MSB-I cells in the presence (●—●) and absence (○—○) of 1 µg/ml IdUrd.

Table 1. Effect of IdUrd on antigen production in MSB-I cells*

<table>
<thead>
<tr>
<th>Incubation time (h)</th>
<th>Control</th>
<th>1 µg/ml</th>
<th>10 µg/ml</th>
<th>20 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSB-I</td>
<td>With IdUrd</td>
<td>Without IdUrd</td>
<td>% positive</td>
<td>% viable</td>
</tr>
<tr>
<td>Low producer</td>
<td>24</td>
<td>48</td>
<td>0.5</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>none</td>
<td>0.5</td>
<td>86</td>
</tr>
<tr>
<td>High producer</td>
<td>24</td>
<td>48</td>
<td>3.0</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>none</td>
<td>2.6</td>
<td>82</td>
</tr>
</tbody>
</table>

* Average of at least three independent experiments.
† Percentage of MDV antigen positive cells on basis of total cell count.
‡ Percentage of viable cells on basis of trypan blue exclusion.

2 to 5% of the cells positive for MDV antigens, were called high producers. Low producer populations were characteristic of cultures passaged more than 80 times in this laboratory, whereas high producer populations were characteristic of cultures passaged fewer than 50 times.

Effect of IdUrd on cell growth

Although IdUrd allowed some cell growth, the rate was substantially less than that of control cultures. As shown in Fig. 1, MSB-I cells doubled at least one time during treatment with IdUrd. The slower growth rate was most likely caused in part by the toxicity of this drug. As shown in Table 1, all concentrations of IdUrd tested were toxic to MSB-I cells; 1 µg/ml afforded the greatest level of viability. Concentrations greater than 10 µg/ml were extremely toxic and resulted in the death of 70% of the cells.

Activation of MDV in MSB-1 cells

Activation of the MDV genome in MSB-1 cells was measured by an increase in cells producing virus specific antigens. Treatment with IdUrd for 24 h resulted in an increase in cells positive for MDV antigens (Fig. 2). If the incubation period was extended to 48 h,
there was a further increase in antigen positive cells (Fig. 2 and 3, and Table 1). Cells treated with 20 μg/ml or 1 μg/ml of IdUrd were examined under the electron microscope for the presence of virus particles. Cultures treated with 20 μg/ml for 48 h showed no increase in the number of cells producing virus. Therefore, IdUrd treatment resulted in an increase in virus antigens without an accompanying increase in virus production. These antigens were initially referred to as EA (Nazerian, 1975). If the IdUrd was removed after 24 h and incubation was continued for an additional 48 h, these cultures then showed an increase in virus particles. Therefore, the presence of 20 μg/ml of IdUrd allowed the synthesis of virus-specific antigens but inhibited at least the assembly of virus-specific antigens. Treatment with 1 μg/ml of IdUrd activated the MDV genome and resulted in the synthesis of both virus antigens and virus particles. In this case the induced antigens most likely represented early and late virus products.

To determine the time required for activation of MDV, MSB-I cells were incubated with IdUrd (1 μg/ml), harvested at various times and prepared for IF. Fig. 2 shows that IdUrd had no immediate effect on antigen expression during the first 12 h of incubation. By 24 h, many antigen positive cells had appeared and by 48 h this level was further increased.

To determine if DNA synthesis was required for activation, MSB-I cells treated with IdUrd were incubated with 0.25 μg/ml ara-C (Table 2). Ara-C, a potent inhibitor of DNA synthesis, substantially reduced the number of antigen positive cells. This reduced number indicated that active DNA synthesis, including incorporation of IdUrd into cellular DNA, was necessary for activation of the MDV genome.

**Effect of cycloheximide on IdUrd activation**

MSB-I cells were treated with cycloheximide in conjunction with IdUrd in tests to determine if the increase in MDV antigen expression required *de novo* protein synthesis. MSB-I cells, treated with IdUrd at time zero, were incubated with 10 μg/ml of cycloheximide at various intervals during a 48 h period. All cultures were harvested at 48 h and examined for MDV antigens. As shown in Fig. 4, the addition of cycloheximide fully inhibited any increase in virus antigens until some time later than 24 h. Such results show
that expression of MDV activation required additional protein synthesis. Cycloheximide had the same qualitative effect on the level of spontaneously producing cells in MSB-1 untreated cultures.

**Effect of IdUrd on high and low producer MSB-1 cells**

Only high producer MSB-1 cells could be activated to any great extent by IdUrd. As shown in Table 1, all concentrations of IdUrd were relatively ineffective in activation of the...
Fig. 4. The effect of cycloheximide on the induction of MDV-associated antigens. MSB-1 cells were grown in the presence (●—●) or absence (○—○) of 1 μg/ml IdUrd. Beginning with time zero, cycloheximide was added at various times to MSB-1 cells. Cultures were harvested after 48 h and prepared for immunofluorescence.

MDV genome of low producer cultures when compared to that of high producer cells. Because the effect of IdUrd on cell viability was essentially the same in low and high producer cultures, the level of MDV induction apparently depended upon an inherent property of the cell population.

DISCUSSION

In the presence of 20 μg/ml IdUrd, the increase in antigen positive cells possibly represented an accumulation of EA, provided this concentration of IdUrd was sufficient to inhibit virus DNA synthesis. This would further be substantiated by the lack of virus particles and the subsequent appearance of these particles after removal of IdUrd. However, workers have shown that some EBV producer human lymphoblastoid cell lines can synthesize both EA and VCA in the presence of IdUrd (10 μg/ml; Gergley, Klein & Ernberg, 1971b; Sugawara, Mizuno & Osato, 1972).

Evidence presented here suggests that IdUrd is most likely incorporated into cellular DNA before the MDV genome can be activated. First, because IdUrd is an analogue of thymidine, it may be implied that IdUrd becomes incorporated into cellular DNA. Second, a minimum of 12 h incubation time with IdUrd is necessary before any expression of the MDV genome is observed; this time corresponded to the normal doubling time of MSB-1 cells. Thirdly, in the presence of ara-C, a potent inhibitor of DNA synthesis, activation by IdUrd is substantially reduced.

It would appear that in MSB-1 cells, the induction of virus antigens occurs in at least two stages. First, IdUrd interacts with MSB-1 cells during the first 12 h of incubation, which most likely results in activation of the MDV genome. Second, virus antigens are synthesized during the next 12 h, and these antigens are sensitive to inhibitors of protein synthesis. The entire process requires at least 24 h before antigen positive cells are significantly increased. This general course of events was observed in MSB-1 cells regardless of the level of virus production.

Although MSB-1 is a producer cell line, it varies in the number of cells producing virus. High producer cultures were easily activated by IdUrd whereas low producer cultures were activated to a lesser degree. A loss of a part of the virus genome during passage in tissue culture or a change in controlling mechanisms could account for these differences. Regard-
less of the reason, the level of spontaneous production may indicate the state of the virus within the cells; and in high producer cultures, more cells may be susceptible to activation.

Activation of the virus genome may involve several changes that cannot be resolved by methods used in this study. A number of cells within the population may already be primed for expression of the virus genome, and IdUrd may cause a selection for those cells already capable of spontaneously producing MDV antigens.

REFERENCES


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