Replication of Feline Herpesvirus Type 1 in Feline T-lymphoblastoid Cells
Taisuke HORIMOTO, Yasushi KAWAGUCHI, Joselito A. LIMCUMPAO, Takayuki MIYAZAWA, Eiji TAKASHASHI, and Takeshi MIKAMI
Department of Veterinary Microbiology, Faculty of Agriculture, The University of Tokyo, Yayoi 1–1–1, Bunkyo-ku, Tokyo 113, Japan
(Received 19 November 1990/Accepted 29 January 1991)

KEY WORDS: feline herpesvirus type 1, T-lymphoblastoid cell.

The herpesviruses are divided into three subgroups (the alpha-, beta-, and gammaherpesviruses) on the basis of their biological properties containing their host ranges, durations of reproductive cycles, and so on [16]. Some herpesviruses are recognized for their abilities to infect lymphocytes. In particular, the gammaherpesviruses have been defined as members which replicate in lymphoblastoid cells in vitro and their infection is frequently arrested either at a pre-lytic stage with persistence and minimum expression of the viral genome or at a lytic stage, causing cell death without production of complete virions [16]. On the other hand, it has been reported that some alphaherpesviruses including herpes simplex virus [5] and equine herpesvirus type 1 [17] also can lytically infect lymphocytes and these infections might be related directly or indirectly to some immunological disorders.

Feline herpesvirus type 1 (FHV-1), an alphaherpesvirus, causes an upper respiratory tract disease in Felidae known as feline viral rhinotracheitis [15]. Earlier studies showed the replication of FHV-1 in cat thymus cell cultures [8] and the isolation of the virus from spleen or mandibular lymph nodes of infected cats [2]. Furthermore, Tham et al. [18] reported the recovery of the virus from lymphocytes of experimentally infected cats using a co-culture method with cat embryo and kidney cells, and suggested the possibility of latent infection in lymphocytes. However, the lytic infection of the virus in lymphocytes is not known either in vitro or in vivo. In the present study, we report on the replication capability of FHV-1 in cultures of T-lymphocytes using feline peripheral blood mononuclear leukocytes (PBLs) and feline T-lymphoblastoid cell lines.

Primary PBLs were prepared from specific-pathogen-free cats according to the previous method [10], stimulated with a mitogen, Concanavalin A. After 3 days non-adherent cells were washed and then cultured in RPMI 1640 growth medium supplemented with 10% heat-inactivated fetal calf serum, 50 μM 2-mercaptoethanol, 2 μg/ml polybrene, 100 units/ml of recombinant human interleukin-2 (rhIL-2), and antibiotics. This culture, which contained more than 95% T-lymphocytes [20], was used as normal feline T-lymphoblastoid (NTL) cells. A feline T-lymphoblastoid cell line, designated as MYA-1 cells, which is free from exogenous retroviruses was also used. Details of the characterization of MYA-1 cells were described elsewhere [10]. Another feline T-lymphoblastoid cell line, FL74, which produced feline leukemia virus [19], was also used in this study. Both MYA-1 and FL74 cells were cultured in the above growth medium except that rhIL-2 was not incorporated in the latter.

FHV-1 C7301 strain [11] with an infectivity titer of 3×10⁷ median tissue culture infectious dose (TCID₃₀) micro-titrated in Crandell feline kidney (CRFK) cells was mixed with 1×10⁷ of T-lymphoblastoid cells in 1.5 ml of Eagle’s minimum essential medium and incubated for 1 hour at 37°C. The cells were then washed three times and suspended in a ratio of 3×10⁵ cells per ml in growth medium. Cell suspensions were cultured at 37°C in a humidified atmosphere of 5% CO₂ in the air and sequentially examined for the appearance of FHV-1 specific antigens and cytopathic effects (CPE) in each of the lymphoblastoid cell cultures.

Indirect immunofluorescence assay (IFA) was performed using FHV-1 infected cat serum and anti-cat IgG FITC-conjugates on glass slides smeared with infected cells to examine the percentages of cells expressing FHV-1 antigens. As shown in Table 1, the rate of positive IFA reactions was clearly different among the three types of cell cultures. About 20% of NTL and 55% of FL74 cells were maximally positive for FHV-1 antigens at 72 hours

Table 1. Indirect immunofluorescence assay for detection of virus-specific antigens in acetone-fixed T-lymphoblastoid cells inoculated with FHV-1

<table>
<thead>
<tr>
<th>Cells</th>
<th>0</th>
<th>6</th>
<th>12</th>
<th>18</th>
<th>24</th>
<th>36</th>
<th>48</th>
<th>72</th>
<th>96</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTL</td>
<td>-</td>
<td>a) ND (b)</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MYA-1</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>FL74</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>++</td>
<td>ND</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

a) Percentages of FHV-1 antigens positive cells were expressed as – (0%), + (1–25%), ++ (26–50%), +++ (51–74%), and ++++ (75–100%).
b) Not determined.
post-inoculation and these values remained until the cells died. On the other hand, specific antigen-positive cells were observed in more than 90% of MYA-1 cells after 36 hours post-inoculation. The CPEs, which included the appearance of some refractile ballooning cells and the increase of intracellular granules, were microscopically observed markedly from 1 day post-inoculation. Thereafter, the death of MYA-1 and FL74 cells soon followed. In the NTL cell culture, CPEs were comparatively unclear although the suppression of growth and death of the cells were seen. These infected cells were clearly distinguished from each of the control mock-infected cells. Fig. 1 shows typical CPEs caused by virus infection of MYA-1 and FL74 cells.

In addition, the titers of replicated viruses in culture supernatants were highest in MYA-1 cells. The highest virus titers reached $10^{10}$, $10^{12}$, and $10^{22}$ TCID$_{50}$/50 µl in NTL, FL74, and MYA-1 cell cultures, respectively, whereas the titer of the remaining input viruses after adsorption was below $10^{5.9}$ (data not shown). However, the titers obtained here do not appear to show complete correlation with the positive cell percentages of FHV-1 antigens in IFA, suggesting that all of the virus antigen-positive cells do not necessarily relate with virus-productive ones, as has been reported in herpes simplex virus type 1 infection in human T-lymphoblastoid cells [5]. Nonetheless, taken together, our observations indicated that FHV-1 can productively infect subpopulations of T-lymphocytes leading to death. We are now in the process of detecting surface antigens on these T-lymphoblastoid cells, especially with respect to CD4 and CD8 antigens.

The growth curve of FHV-1 on MYA-1 cell culture was determined to examine fully the replication cycle in the cells and the influence to cell growth with the virus infection (Fig. 2). After 3 passages of FHV-1 in MYA-1 cells the virus was inoculated at a multiplicity of infection (MOI) of 3 TCID$_{50}$/cell, and the replicated viruses were harvested both from the extra- and intracellular phases at the times indicated in Fig. 2. The supernatants, after pelleting infected cells from suspension cultures, were used as extracellular phase samples. On the other hand, infected cells were washed three times, resuspended in RPMI 1640, and ultra-sonicated on ice. The supernatants obtained after centrifugation were then used as intracellular phase samples. Viable cells were counted on trypan blue stained preparations. The maximum infectivity titers of extra- and intracellular viruses were obtained at 36–48 hours post-inoculation (hpi) and 24–36 hpi, respectively. The growth cycle in MYA-1 cells represented here was rapid like those in monolayer cultures of feline lung, CRFK, and feline cats whole fetus (fcwf-4) cells reported previously [6]. The numbers of viable FHV-1-infected cells decreased after 24 hpi, although mock-infected cells increased by 72 hpi, indicating that the death of cells were caused by virus replication.

Next, we performed analysis of FHV-1 proteins synthesized in MYA-1 cells using immunoblotting assay and anti-FHV-1 monoclonal antibodies [7], and compared them with those in CRFK cells to examine the differences...
in virus protein synthesis between these two types of cells. No differences in molecular weights of the three major FHV-1 glycoproteins were observed between them (data not shown), suggesting the similarity of the virus protein synthesis including glycosylation.

Recent studies suggested that the human herpesvirus group might be a potential cofactor involved in the pathogenesis of acquired immunodeficiency syndrome (AIDS) and of related disorders which are caused primarily by the human immunodeficiency virus (HIV-1). Herpes simplex virus type 1 (HSV-1) [3, 4, 12, 13, 14], varicella-zoster virus [3], human cytomegalovirus [1], and human herpesvirus type 6 (HHV-6) [9] or cloned DNA fragments containing immediate-early genes of these viruses are capable of trans-activating the long terminal repeat (LTR) containing essential promoter sequences for HIV-1 gene expression. Furthermore, HSV-1 and HHV-6 can infect T-lymphocytes, which are also the target cells for HIV-1 infection, and these herpesviruses, consequently, might be effective cofactors in the pathogenesis of human AIDS in vivo. In fact, dual infection of HHV-6 and HIV-1 causes a more rapid loss of CD4+ T-lymphocytes [9]. Feline immunodeficiency virus (FIV) infection, though thought to induce immunodeficiency in cats, is one of the most promising animal models for AIDS in human. We have observed the dual infection by FIV and FHV-1 of PBLs and MYA-1 cells and the activation of the LTR of FIV by FHV-1 infection (Kawaguchi et al., submitted). Therefore, FIV and FHV-1 system may contribute us to the elucidation of the interactions between retroviruses and herpesviruses as they relate to the pathogenesis of AIDS.

ACKNOWLEDGEMENTS. This work was supported by grants from the Ministry of Education, Science and Culture, Japan.

REFERENCES