Stimulation of Lymphokines in Jurkat Cells Persistently Infected with Vaccinia Virus

KATHLEEN A. STELLRECHT,† KIRK SPERBER, BEATRIZ G.-T. POGO

Department of Neoplastic Diseases and Division of Clinical Immunology,
Mount Sinai School of Medicine, New York, New York 10029-6574

Received 7 October 1991/Accepted 18 December 1991

The response of the human CD4+ T-cell line Jurkat to infection with vaccinia virus was investigated. Virus titers peaked approximately 3 to 4 days after infection, while cell growth paralleled that of uninfected cells, indicating that growth rates were not appreciably affected by viral infection. Results from plaque assays and fluorescence-activated cell sorter (FACS) analyses of virus antigens demonstrated that a persistent infection in which the percentage of infected cells and the virus titers fluctuated from passage to passage was established. Further characterization of the persistent infection revealed that the virus influences cellular functions. Induction of interleukin-2 (IL-2) and IL-2 receptor α (IL-2Rα) in Jvac cells was shown by enzyme-linked immunosorbent assay and FACS analysis, respectively. Hybridization of cellular RNA with cloned probes confirmed the increased IL-2 expression and demonstrated that Jvac cells also expressed more IL-6 but not gamma interferon (IFN-γ) or IL-1β. Dual-antibody staining and FACS analysis for vaccinia virus antigens and IL-2Rα indicated that IL-2Rα expression was restricted to the infected cells. Jvac cells were also resistant to superinfection, an additional proof that persistent infection elicited phenotypic changes in the cell population.

MATERIALS AND METHODS

Virus, cells, and reagents. The IHD-W strain of vaccinia virus was propagated and assayed in L-cell monolayers (10). Jurkat cells (a gift of Miriam Sieckevitz) were suspended in RPMI (GIBCO) medium supplemented with 10% fetal calf serum (Reheis Chemical Co.) and penicillin-streptomycin (GIBCO). In some experiments, cells were stimulated with 2% Bacto-phytohemagglutinin M (PHA) (Difco Laboratories, Detroit, Mich.) and/or 50 ng of phorbol 12-myristic 13-acetate (PMA) per ml for 24 h.

To initiate viral infection in Jurkat cells, 5 × 10⁶ cells were resuspended in 1.0 ml of tissue culture medium and adsorbed with 5 PFU of virus per cell for 1 h at room temperature with gentle shaking. Unadsorbed virus was removed by centrifugation at 400 × g for 5 min at room temperature, and the cell pellet was resuspended in 25 ml of fresh medium. Cell viability was determined by trypan blue exclusion, and infectivity was determined by plaque assays in L cells (10). Persistently infected cells, designated Jvac, were passaged one or two times a week by being diluted 1:5 in fresh medium.

ELISA for IL-2 production. Jurkat and Jvac cells were cultured for 1 week at 37°C in a 5% CO₂ atmosphere, at which time concentrations reached 1 × 10⁶ to 2 × 10⁶ cells per ml. Cultures were centrifuged at 400 × g, and the supernatant was used for IL-2 assay. To measure IL-2 production during the primary infection, cells were infected and resuspended to concentrations of 2 × 10⁶ cells per ml. Samples were taken at the times indicated in Table 2. The Inter-Test-2 human IL-2 enzyme-linked immunosorbent assay (ELISA) kit (Genzyme, Boston, Mass.) was used to measure IL-2 levels in the culture media according to the methods described by the manufacturer. All samples were assayed in duplicate or triplicate.

Fluorescence-activated cell sorter (FACS) analysis and antibodies. Polyclonal anti-vaccinia virus antiserum was obtained from rabbits inoculated with vaccinia virus and labeled with biotinylated goat anti-rabbit immunoglobulin antisera. An anti-TAC (IL-2Rα) monoclonal antibody (Becton-Dickinson, Rutherford, N.J.) was fluorescein isothiocyanate (FITC) conjugated. Intracytoplasmic staining and

* Corresponding author.
† Present address: Department of Clinical Microbiology, Albany Medical Center Hospital, Albany, NY 12208.
analysis on an Epic S C FACS (Coulter Scientific, Hialeah, Fla.) were performed as previously described (28).

**RNA extraction.** Approximately 10^7 cells were incubated with 1 mg of proteinase K per ml and 0.5% Sarkosyl at 37°C overnight, and then nucleic acid was extracted with phenol and chloroform. The resulting nucleic acid extract was treated with DNase I as previously described (24). The amount of RNA was estimated by extinction at 260/280 nm, and the removal of DNA was confirmed by measurements on a mini-fluorometer (Hoefer Scientific Instruments, San Francisco, Calif.).

**Slot blot hybridization.** Serial dilutions of 20, 10, 5, and 2.5 μg of cellular RNA were applied to nylon filters by using a slot blot filtration apparatus (Schleicher & Schuell, Keene, N.H.). Hybridization was carried out by using nick-translated probes under the conditions described by Thomas (30). Plasmid probes were labeled with 35S-nucleotides by nick translation by the procedure of Ribgy et al. (27). The following plasmids (gifts from Lloyd Mayer, Mount Sinai Medical Center, New York, N.Y.) were used: pGEMZ-2G, a 1.5-kb BamHI fragment of the human IL-1β gene; pLW81, a 570-bp PstI fragment from the human IL-2 gene; pGEMZ-IL6, a PstI 1.3-kb fragment from the human IL-6 gene; and pGEMZ gamma-IFN-5 which contains a fragment from the human gamma interferon (IFN-γ) gene. For comparison, expression of a housekeeping gene was evaluated by probing with pGEM-3-actin-5, a PstI-XbaI 600-bp fragment of the actin gene. Autoradiographs were scanned with an LKB Ultra Scan laser densitometer. Radiolabeled probes were removed from nylon membranes by immersion in 50% formamide for 1 h at 65°C (30).

**RESULTS**

**Infection of Jurkat cells with vaccinia virus.** Cell growth and virus infectivity were determined during the acute phase of infection in Jurkat cells infected with vaccinia virus. As shown in Fig. 1, infected cells grew at the same rate as mock-infected cells, in which there was a steady increase until a stationary phase was reached about 10 days postinfection (p.i.). Virus titers, on the other hand, peaked 2 to 3 days after infection and again 10 days p.i. Furthermore, it was evident that the cells survived the primary infection and that viral infectivity was maintained after subsequent weekly passages. Results from FACS analysis of vaccinia virus antigens correlated with those of the plaque assay, indicating a peak of viral replication between 72 and 96 h p.i., at which time 60% of the cells were infected (data not shown). Determination of virus infectivity, monitored weekly, demonstrated that virus titers fluctuated considerably, ranging from undetectable to approximately 7.63 log units/10^6 cells in the experiment whose results are shown in Table 1. In other cell lines, titers of 1.5 × 10^6 PFU/10^6 cells were recorded. The data also suggested that Jvac cells are not a homogeneous population, since the percentage of infected cells varied (Tables 1 and 2).

**Characterization of Jvac cells.** Changes in cellular function were investigated during primary infection and at various passages in Jvac cells. It appears that during the acute phase of infection the virus had little effect on the activation or differentiation of Jurkat cells. ELISAs demonstrated that during the first days of infection there was no increase in the level of IL-2 in the supernatants from Jvac cells compared with the level in supernatants from control cells. Similar results were seen when cells were stained with FITC-labeled anti-TAC antibodies and evaluated for IL-2Rα expression by FACS analysis. At 48 and 72 h p.i., the percentages of stained cells were insignificant (data not shown).

Further characterization of Jvac cells indicated that the establishment of the persistent infection was associated with changes in cellular functions as early as 96 h p.i. (Table 2). Production of IL-2 by Jvac cells was demonstrated at various passages by ELISA of the culture media. FACS analysis indicated that the cells expressed IL-2Rα, although this expression appeared to be more variable. To correlate IL-2Rα production with infectivity, dual staining with both anti-vaccinia virus and anti-TAC antibodies and subsequent FACS analysis were performed. The staining pattern of Jvac cells was consistent with restriction of FITC-labeled anti-TAC staining to the infected cells, suggesting that IL-2Rα expression was dependent on the presence of the virus (Fig. 2).

RNAs from Jurkat and Jvac cells, as well as from PHA-PMA-stimulated Jurkat cells, were analyzed for gene expression by slot blot hybridization with radiolabeled probes for IL-1β, IL-2, IL-6, and IFN-γ genes. Actin expression was
TABLE 1. Evolution of infectivity in J vac cells

<table>
<thead>
<tr>
<th>Passage</th>
<th>PFU (log)</th>
<th>Dead cells (%)</th>
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<tbody>
<tr>
<td>1</td>
<td>6.29</td>
<td>11.4</td>
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<tr>
<td>5</td>
<td>6.19</td>
<td>13.6</td>
</tr>
<tr>
<td>12</td>
<td>4.74</td>
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<td>23</td>
<td>2.43</td>
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<tr>
<td>72</td>
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</table>

* Results are representative of one J vac cell line.

a Log of the PFU per 10⁶ cells.

b ND, not determined.

also investigated as an internal control. The histogram in Fig. 3 demonstrates increased IL-2 expression in J vac cells, confirming the ELISA results. Furthermore, an increase in IL-6 mRNA but not in IFN-γ mRNA was evident in J vac cells, while the levels of both mRNAs were elevated in PHA-PMA-stimulated cells. Finally, there was no increase in the expression of the monokine IL-1β or of actin, as expected.

**Resistance of J vac cells to superinfection.** A phenotypic characteristic of persistently infected cells is resistance to superinfection (1, 11, 23, 25). The effect of superinfection on J vac cells was therefore investigated. Infected cells were challenged with 5 PFU of virus per cell. The cell line chosen for these experiments was one in which virus infectivity was undetectable at that time. As shown in Fig. 4, there was a modest increase in viral infectivity at 72 and 96 h p.i., but this increase was much lower than the level seen in primary infections of Jurkat cells. By 168 h, viral infectivity was back to the initial low levels, indicating that incoming virus was able to replicate but to a lesser extent than Jurkat cells infected with the same multiplicity.

**DISCUSSION**

Vaccinia virus is cytocidal to most cells; however, under certain conditions, persistent or latent infections have been demonstrated (23–25). In this report, we describe the establishment of a persistent infection in T lymphocytes. Evaluation of the acute phase of infection demonstrated that the growth of the infected cells paralleled that of control cells, that these cells survived the infection, and that a persistent infection was established (Fig. 1). Although virus titers fluctuated considerably from one passage to the next, infectivity through 75 weeks of infection was demonstrated (Table 1).

The mechanism(s) responsible for the establishment and maintenance of the persistent infection is not clear but may be the result of the cells replicating at a slightly greater rate than the virus. Indeed, not all cells are infected at one time (Table 2). In addition, interferon and other viral inhibitors may play a role in maintaining the persistent infection, as seen in other systems (4, 26, 29, 33). Although it appears that J vac cells did not express IFN-γ, production of IFN-α and IFN-β in vaccinia virus-infected FEL cells has been de-
binding of possible vaccinia mitogenlike activity.

Indeed, vaccinia virus-infected cells did not express IFN-γ (Fig. 3). By contrast, vaccinia virus-infected cells did not express IFN-γ (Fig. 3). This selective stimulation is an unusual finding, because T-cell activation is associated with the expression of all these genes (32).

Antigenic stimulation of T cells is associated with the induction of more than 100 genes (32). Genes which are expressed during this period include those encoding lymphokines and surface receptors necessary to further potentiate the immune response. This activation can mimic the binding of many mitogens, including PHA and PMA (9). It is possible that vaccinia virus may mediate its effects through a mitogenlike activity. The discovery that poxviruses codified for a viral growth factor and for other virokinines (18, 31) indicated that they are able to exert extracellular effects. Indeed, it has been demonstrated that viral growth factor induces cell proliferation both in vitro and in vivo (7, 19).

However, assays for mitogenic activity of concentrated conditioned media from J vac cells were consistently negative (unpublished data).

The significance of the effects of vaccinia virus on cell differentiation is not clear at the moment, but the effects imply the presence of trans-acting factors induced by the virus. Viral transactivator genes and the ability of their protein products to influence the expression of cellular genes have been described for some DNA-transforming viruses (3, 12, 16). Vaccinia virus trans-acting factors have been demonstrated (17); however, their ability to influence cellular genes has yet to be shown. Alternatively, vaccinia virus could turn on cellular trans-acting factors, either directly or by blocking an inhibitor of these factors. Indeed, the vaccinia virus genome encodes for protein kinases (14). Together with the fact that inhibitors of trans-acting factors are inactivated by phosphorylation (6, 13), a role for vaccinia virus in this context can be postulated. Investigations to identify the cellular and viral genes responsible for these virus-host cell responses are under way.

Finally, it is worth mentioning that, in addition to the effects on cellular functions, changes in the viral population have been recorded as previously described (20, 22). Restriction enzyme analysis of viral DNA from J vac cells demonstrated the emergence of deletion mutants during the persistent infection (unpublished data).
ACKNOWLEDGMENTS
We acknowledge A. Pizzimenti for performing the FACS analysis and Dolores Klaft for excellent secretarial help.
This project was funded by NIH grant CA29262 and by grants from the T. J. Martell Foundation and the Chemotherapy Foundation.

REFERENCES