JOURNAL OF VIROLOGY, Apr. 1992, p. 2046–2050
0022-538X/92/042046-05$02.00/0

Vol. 66, No. 4

Stimulation of Lymphokines in Jurkat Cells Persistently Infected with Vaccinia Virus

KATHLEEN A. STELLRECHT,[1]† KIRK SPERBER,[2] AND BEATRIZ G.-T. POGO[1]*

Department of Neoplastic Diseases[1] and Division of Clinical Immunology,[2] 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 J_{vac} 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 J_{vac} 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. J_{vac} cells were also resistant to superinfection, an additional proof that persistent infection elicited phenotypic changes in the cell population.

The abilities of some viruses or their gene products to alter in vitro and in vivo cellular functions are well documented. Some examples of these effects are inhibition of growth hormone by lymphocytic choriomeningitis virus (21), altered immunoglobulin synthesis by measles and influenza viruses (8), activation of interleukin-2 (IL-2) and IL-2 receptor α (IL-2Rα) genes by the Tax protein of human T-cell leukemia virus type 1 (5, 15), and differentiation of erythroid leukemia cells by vaccinia virus (20, 23–25).

Despite the cytocidal nature of vaccinia virus, persistent infections have been established in two cell lines, murine Friend erythroleukemia (FEL) cells and human erythroleukemia (K562) cells (20, 23–25). Investigations of these persistent infections demonstrated that infected erythroleukemia cell lines display high levels of spontaneous differentiation after serial passage in vitro (20, 23–25). Northern (RNA) blot hybridization of extracts from infected K562 cells indicated that there is increased expression of fetal hemoglobin, mainly of the G-gamma type (25). Studies of the persistently infected FEL cells demonstrated that these cells are less tumorigenic than the parental cells, resist superinfection, and show chromosomal abnormalities (20, 23).

The mechanism responsible for the effects of vaccinia virus on erythroleukemia cells is still unclear, mainly because knowledge of the regulatory mechanisms for erythroid differentiation is limited. On the other hand, processes involved in T-lymphocyte differentiation have been extensively studied (for a review, see reference 32). Hence, studies of the effects of vaccinia virus on T-cell functions can be studied in greater depth at the molecular level. This report describes the establishment and characterization of Jurkat cells persistently infected with vaccinia virus and some of the changes in cellular functions that occurred. It is expected that this model system will provide new knowledge about the mechanisms by which vaccinia virus exerts its effects on cellular functions.

* Corresponding author.
† Present address: Department of Clinical Microbiology, Albany Medical Center Hospital, Albany, NY 12208.

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 Siekevitz) 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, 5×10^6 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 $400 \times g$ 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 J_{vac}, were passaged one or two times a week by being diluted 1:5 in fresh medium.

ELISA for IL-2 production. Jurkat and J_{vac} cells were cultured for 1 week at 37°C in a 5% CO_2 atmosphere, at which time concentrations reached 1×10^6 to 2×10^6 cells per ml. Cultures were centrifuged at $400 \times g$, 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 2×10^5 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

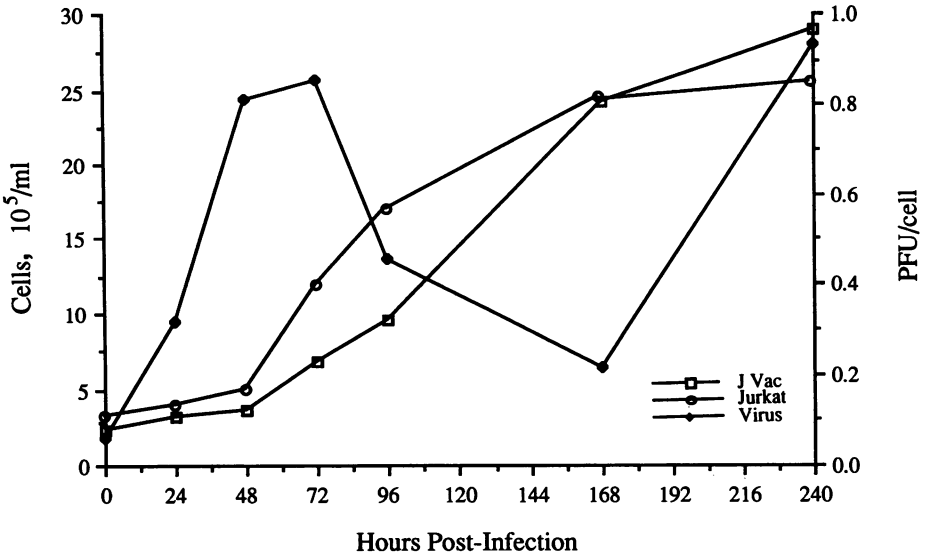

FIG. 1. Effect of vaccinia virus infection on Jurkat cells. Cells were either infected with 5 PFU of vaccinia virus per cell (□) or mock infected (○). Samples were taken to determine cell growth and virus titers (◆) at the times indicated. The graph represents results from one of five different experiments performed.

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 nytran 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 ^{35}S-nucleotides by nick translation by the procedure of Rigby et al. (27). The following plasmids (gifts from Lloyd Mayer, Mount Sinai Medical Center, New York, N.Y.) were used: pGEM3Z-2G, a 1.5-kb *Bam*HI fragment of the human IL-1β gene; pLW81, a 570-bp *Pst*I fragment from the human IL-2 gene; pGEM3Z-IL6, a *Pst*I 1.3-kb fragment from the human IL-6 gene; and pGEM3Z 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 *Pst*I-*Xba*I 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 1.5×10^8 PFU/10^6 cells were recorded. The data also suggested that J_{vac} cells are not a homogeneous population, since the percentage of infected cells varied (Tables 1 and 2).

Characterization of J_{vac} cells. Changes in cellular function were investigated during primary infection and at various passages in J_{vac} 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 J_{vac} 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 J_{vac} 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 J_{vac} 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 J_{vac} 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 J_{vac} 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[a]

Passage	PFU (log)[b]	Dead cells (%)
1	6.29	11.4
5	6.19	13.6
12	4.74	11.1
18	1.00	9.2
23	2.43	ND[c]
28	5.71	16.4
32	3.30	11.4
37	5.77	2.5
41	6.69	17.0
45	5.47	15.3
50	5.69	15.8
54	6.32	34.2
58	7.63	18.1
63	5.38	9.6
67	5.41	27.7
72	6.36	55.6

[a] Results are representative of one J_{vac} cell line.
[b] Log of the PFU per 10^6 cells.
[c] 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.

TABLE 2. Evolution of infectivity and gene expression in J_{vac} cells[a]

Condition	% Infected	% IL-2R positive	IL-2 level (ng/ml)[b]
96 h p.i.	61.36	8.5	3.5
240 h p.i.	38.11	16.75	7.0
Passage			
1	53.82	ND	6.5
6	15.81	10.85	8.0
7	21.46	1.14	11.5
8	7.20	8.20	2.6
9	58.12	7.60	9.6
10	65.23	17.15	10.8
13	48.17	5.15	8.5
16	3.90	13.40	4.8
18	5.50	3.95	3.1
22	4.50	5.69	0.9
35	11.98	10.85	2.9
53	19.84	21.38	3.7
64	3.92	4.28	1.0
Control (SD)	3.82 (1.52)	3.15 (1.41)	0.3
PHA (SD)	ND (ND)	20.19 (4.27)	8.22 (3.54)

[a] ND, not determined.
[b] Level in culture medium from 10^6 cells per ml after 1 week in culture.

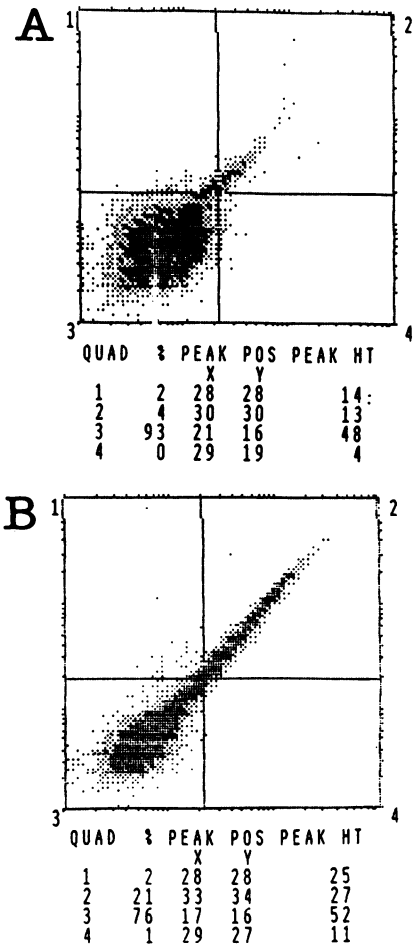

FIG. 2. IL-2Rα expression in J_{vac} cells as demonstrated by dual staining and FACS analysis. Polyclonal biotinylated anti-vaccinia virus (*y* axis) and FITC-conjugated anti-TAC (*x* axis) antisera were used to stain both Jurkat (A) and J_{vac} (B) cells.

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-

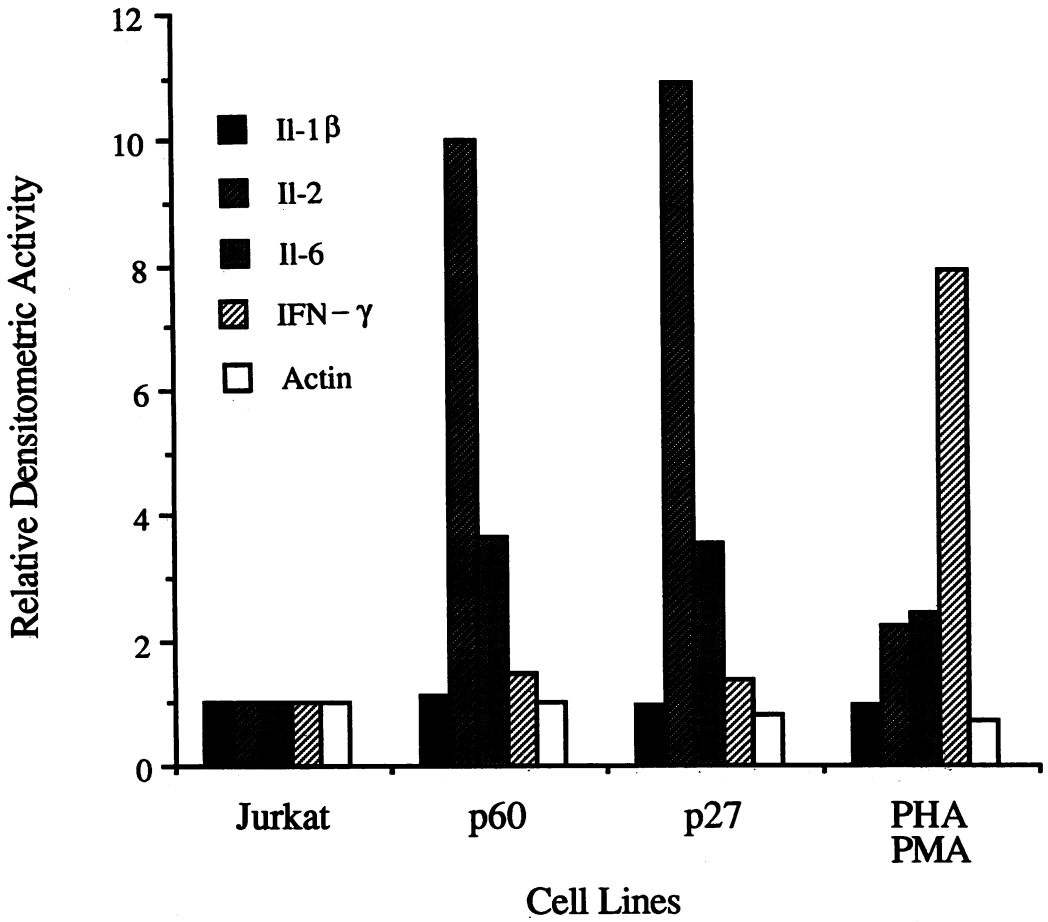

FIG. 3. Hybridization of cellular RNA to labeled genes. Autoradiographs of slot blots of cellular RNA from Jurkat, J_{vac} (passages 27 and 60), and PHA-PMA-stimulated Jurkat cells were scanned with an LKB Ultra Scan laser densitometer. Densitometric activity is presented as a fraction of Jurkat cell values.

scribed (22). Alternatively, the persistent infection may be related to the heterogeneity of the cell population. It is evident that J_{vac} cells are not a homogeneous population (Fig. 2). Selective conditions for long-term virus and cell survival are created when a cell population with a range of responses toward the virus is provided (11).

Interestingly, it appears that the persistent infection influences cellular functions. In addition to resistance to superinfection (Fig. 4), vaccinia virus induced T-cell differentiation, as demonstrated by the increase of IL-2, IL-2Rα, and IL-6 expression in J_{vac} cells (Table 2 and 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 virokines (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).

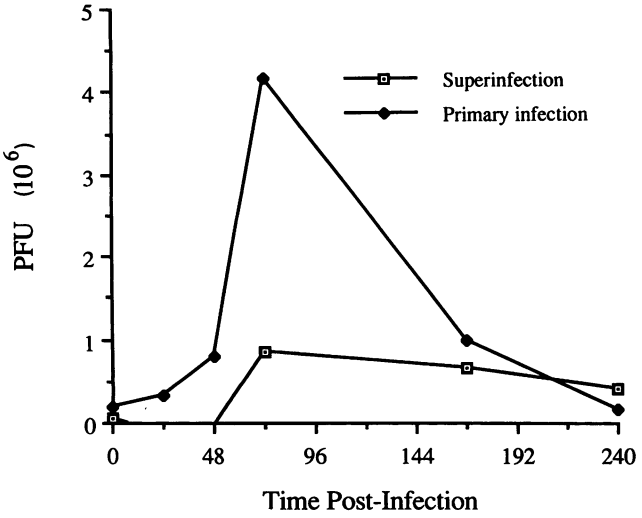

FIG. 4. Superinfection of cells persistently infected with vaccinia virus. Jurkat and J_{vac} cells were inoculated with 5 PFU of virus per cell. Virus infectivity was determined at the times indicated.

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

1. **Ahmed, R., W. M. Canning, R. S. Kauffman, A. H. Sharpe, J. V. Hallum, and B. N. Fields.** 1981. Role of the host cell and persistent viral infection: co-evolution of L cells and reovirus during persistent infection. Cell **25**:325–332.
2. **Ballard, D. W., E. Böhnlein, J. W. Lowenthal, Y. Wano, B. R. Franza, and W. C. Greene.** 1988. HTLV-1 tax induces cellular proteins that activate the κB element in the IL-2 receptor α gene. Science **241**:1652–1655.
3. **Bishop, J. M.** 1991. Molecular themes in oncogenesis. Cell **64**:235–248.
4. **Blackmore, R. V., and H. R. Morgan.** 1967. Self-limiting infection with herpes simplex virus in cell culture. Acta Virol. **11**:1–12.
5. **Böhnlein, E., J. W. Lowenthal, M. Siekevitz, D. W. Ballard, B. R. Franza, and W. C. Greene.** 1988. The same inducible nuclear proteins regulate mitogen activation of both the interleukin-2 receptor-alpha gene and type 1 HIV. Cell **53**:827–836.
6. **Buchkovich, K., L. A. Duffy, and E. Harlow.** 1989. The retinoblastoma protein is phosphorylated during specific phases of the cell cycle. Cell **58**:1097–1105.
7. **Buller, R. M. L., S. Chakrabarti, B. Moss, and T. Fredrickson.** 1988. Cell proliferative response to vaccinia virus is mediated by VGF. Virology **164**:182–192.
8. **Casali, P., G. P. A. Rice, and M. B. A. Oldstone.** 1984. Viruses disrupt functions of human lymphocytes. J. Exp. Med. **159**: 1322–1337.
9. **Crabtree, G. R.** 1990. Contingent genetic regulatory events in T-lymphocyte activation. Science **243**:355–361.
10. **Dales, S.** 1963. Uptake and development of vaccinia virus in strain L cells followed with labeled viral deoxyribonucleic acid. J. Cell Biol. **18**:51–72.
11. **de la Torre, J. C., E. Martínez-Salas, J. Diez, A. Villaverde, F. Gebauer, E. Rocha, M. Dávila, and E. Domingo.** 1988. Coevolution of cells and viruses in a persistent infection of foot-and-mouth disease virus in cell culture. J. Virol. **62**:2050–2058.
12. **Dyson, N., K. Buchkovich, P. Whyte, and E. Harlow.** 1989. Cellular proteins that are targeted by DNA tumor viruses for transformation. Int. Symp. Princess Takamatsu Cancer Res. Fund **20**:191–198.
13. **Ghosh, S., and D. Baltimore.** 1990. Activation *in vitro* of NF-kappa B by phosphorylation of its inhibitor I kappa B. Nature (London) **344**:678–682.
14. **Goebel, S. J., G. P. Johnson, M. E. Perkus, S. W. Davis, J. P. Winslow, and E. Paoletti.** 1990. The complete DNA sequence of vaccinia virus. Virology **179**:247–266.
15. **Hoyos, B., D. W. Ballard, E. Böhnlein, M. Siekevitz, and W. C. Greene.** 1989. Kappa B-specific DNA binding proteins: role in the regulation of human interleukin-2 gene expression. Science **244**:457–460.
16. **Hunter, T.** 1991. Cooperation between oncogenes. Cell **64**:249–270.
17. **Keck, J. G., C. J. Baldick, Jr., and B. Moss.** 1990. Role of DNA replication in vaccinia virus gene expression: a naked template is required for transcription of three late transactivator genes. Cell **61**:801–809.
18. **Kotwal, G. J., A. W. Hugin, and B. Moss.** 1989. Mapping and insertional mutagenesis of a vaccinia virus gene encoding a 13,800-Da secreted protein. Virology **171**:579–587.
19. **Lai, A. C.-K., and B. G.-T. Pogo.** 1989. Attenuated deletion mutants of vaccinia virus lacking the vaccinia growth factor are defective in replication *in vivo*. Microb. Pathog. **6**:219–226.
20. **Obom, K. M., S. W. Popple, J. G. Holland, B. G.-T. Pogo, and C. Friend.** 1986. Vaccinia virus DNA sequences in the nucleus of persistently infected Friend erythroleukemia cells. Virus Res. **5**:221–234.
21. **Oldstone, M. B. A., Y. N. Sinha, P. Blount, A. Tihon, M. Rodriguez, R. von Wedel, and P. W. Lampert.** 1982. Virus induced alterations in homeostasis: alterations in differentiated functions of infected cells *in vivo*. Science **218**:1125–1127.
22. **Paez, E., and M. Esteban.** 1988. Stability of vaccinia virus DNA during persistent infections: accumulation of left-end deletions and of tandem repeats at both ends of the viral genome and prevention by interferon. Virology **163**:145–154.
23. **Pogo, B. G.-T., and C. Friend.** 1982. Persistent infection of Friend erythroleukemia cells with vaccinia virus. Proc. Natl. Acad. Sci. USA **79**:4805–4809.
24. **Pogo, B. G.-T., A. C.-K. Lai, J. G. Holland, and C. Friend.** 1988. Differences in the susceptibility of human blood cell lines to vaccinia virus. Intervirology **29**:11–20.
25. **Pogo, B. G.-T., A. C.-K. Lai, M. E. Joesten, M. E. Roysten, and D. Holloway.** 1991. Changes in cell gene expression in human leukemia cells persistently infected with vaccinia virus. Virus Res. **19**:131–138.
26. **Ramseur, J. M., and R. M. Friedman.** 1977. Prolonged infection of interferon-treated cells by vesicular stomatitis virus: possible role for temperature-sensitive mutants and interferon. J. Gen. Virol. **37**:523–533.
27. **Rigby, P. W., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labelling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol. **113**:237–251.
28. **Sperber, K., S. K. Goswami, E. Gollub, L. Mayer, and Z. Maron.** 1991. Mucus secretagogue production by a human macrophage hybridoma. J. Allergy Clin. Immunol. **87**:490–498.
29. **Tang, J. L., M. Yamamoto, S. Sakuna, R. Mori, and A. Nagayama.** 1988. Persistent infection with herpes simplex virus type 1 in an Ia antigen-positive murine macrophage cell line. Microbiol. Immunol. **32**:363–374.
30. **Thomas, P. S.** 1983. Hybridization of denatured RNA transferred or dotted to nitrocellulose paper. Methods Enzymol. **100**:255–266.
31. **Twardzik, D. R., J. P. Brown, J. E. Ranchalis, G. J. Todaro, and B. Moss.** 1985. Vaccinia virus-infected cells release a novel polypeptide functionally related to transforming and epidermal growth factors. Proc. Natl. Acad. Sci. USA **82**:5300–5304.
32. **Ullman, K. S., J. P. Northrop, C. L. Verweij, and G. R. Crabtree.** 1990. Transmission of signals from the T lymphocyte antigen receptor to the genes responsible for cell proliferation and immune function: the missing link. Annu. Rev. Immunol. **8**:421–452.
33. **Yamada, A., M. Tsurudome, M. Hishiyama, and Y. Ito.** 1984. Abortive infection of mumps virus in murine cell lines. J. Gen. Virol. **65**:973–980.