Antiviral Research, 17 (1992) 79–89

AVR 00519

Differential effects of human recombinant interferons on the expression of two early gene products of Epstein-Barr virus

Bodil Lidin and Eddie W. Lamon

Birmingham V.A. Medical Center and Departments of Surgery, Microbiology and Comparative Medicine and the Comprehensive Cancer Center, University of Alabama at Birmingham, University Station, Birmingham, Alabama, U.S.A.

(Received 15 April 1991; revision accepted 19 June 1991)

Summary

Two human Burkitt's lymphoma (BL) cell lines, Raji and Daudi, have been previously characterized as resistant and sensitive, respectively, to the anti-Epstein-Barr virus (EBV) effects of human leukocyte interferon. These cells are equally susceptible to P3HR-1 EBV superinfection as determined by EBV early antigen (EA) expression. The cell lines were pretreated with human recombinant interferons α_2, β, or γ and subsequently superinfected with P3HR-1 EBV. Their expression of two distinct EBV early gene products was evaluated by fluorescence microscopy. Monoclonal antibodies to the diffuse (EA-D) and restricted (EA-R) components of the EA complex were used to determine the number of cells expressing each of these antigens in the treated cell lines. As previously described with human leukocyte interferon, EA-D expression in Raji cells was relatively resistant to interferon-α_2 pretreatment. Also, EA-D expression in Daudi cells was relatively sensitive. However, interferon α_2 pretreatment produced an opposite pattern with respect to the expression of EA-R in these two cell lines; Raji cells were sensitive and Daudi cells relatively resistant. Interferon β had the most uniformly effective anti-EBV activity on both cell lines; <15 U/ml produced 50% inhibition of both antigens in both cell lines. EA-D expression in both cell lines was sensitive to interferon-γ pretreatment and EA-R was resistant. These data suggest that different gene products of EBV are independently regulated by interferons based on at least three factors: (1) the host cell, (2) the type of interferon and (3) the affected gene product.

Correspondence to: Bodil Lidin, Birmingham V.A. Medical Center, University of Alabama at Birmingham, University Station, Birmingham, AL 35294, U.S.A.

Epstein-Barr virus; Interferon α_2; Interferon β; Interferon γ; Early gene product

Introduction

Epstein-Barr virus (EBV) is a B-cell-trophic herpes virus that is the known cause of infectious mononucleosis (Henle et al., 1968) and is associated with, sometimes lethal, lymphoproliferative diseases under circumstances of immunosuppression secondary to AIDS (Groopman et al., 1986), genetic immunodeficiencies (Purtilo et al., 1982) or iatrogenic induction for transplantation (Hanto et al., 1981). EBV is also strongly associated with African Burkitt's lymphoma (BL) and nasopharyngeal carcinoma (NPC) (zur Hausen et al., 1970; Nonoyama et al., 1973). Most BL cell lines contain resident EBV genome(s) (Adams, 1979). These latent genomes are stably maintained in the cells and can be activated by superinfection with the P3HR-1 virus (Klein et al., 1972). An abortive productive cycle is induced and early antigens (EA) are expressed. However, the cells do not go on to produce infectious virus. The EA expressed in these cells has been shown to vary with the number of resident viral genomes (Zur Hausen et al., 1973; Lidin et al., 1982). Thus a major proportion of EBV gene expression following superinfection by P3HR-1 virus is due to a transactivation of resident viral genomes (Lidin et al., 1982a; Lieberman et al., 1986; Takada et al., 1986).

Interferons are cytokines that induce a relative state of resistance to viral infection in cells that have been exposed to them prior to infection. Three major categories of interferons have been characterized: α corresponds to leukocyte interferon, β to fibroblast interferon and γ to immune interferon (Joklik, 1985). Susceptibility of cells to the antiviral state induced by interferon varies according to the cells and virus under study. Previous studies have shown that human leukocyte interferon can suppress EBV early gene expression in BL cell lines (Adams et al., 1975b). However, the inhibition of the EBV-determined diffuse early antigen (EA-D) by interferon was found to depend on the cell line being infected by P3HR-1 EBV (Adams et al., 1975a). For example, Raji cells were found to be relatively resistant and Daudi cells relatively sensitive to the antiviral effects of interferon (Adams et al., 1975a). A somatic cell hybrid between these Raji and Daudi cells was found to have an interferon sensitivity that was intermediate to the two parental cell lines (Lidin and Lamon, 1982). These studies were conducted with human serum containing antibodies to the diffuse component of the EA complex (EA-D) and human leukocyte interferon obtained from stimulated buffy-coat leukocytes (Cantell and Phylälä, 1973). The latter was probably mostly α but may have contained some interferons β and γ. In the present study we evaluated human recombinant interferons α, β and γ for their capacity to inhibit EBV EA expression. Monoclonal antibodies were used to detect two specific early gene products designated diffuse (EA-D) and restricted (EA-R) following P3HR-1 EBV infection. Two BL cell lines, Raji

and Daudi were used in these experiments. These two cell lines have been shown to be equally susceptible to P3HR-1 EBV superinfection in terms of EA expression. As indicated above, Raji cells had previously been characterized as resistant to the anti-EBV effects of human leukocyte interferon, whereas Daudi cells were found to be sensitive. Kure et al. (1986) have shown that recombinant interferons α and γ inhibited EBV infection. Normal B lymphocytes and the EBV genome-negative BL cell line BJAB became resistant to B958 EBV infection following pretreatment with these recombinant interferons. In their studies interferon α was a more efficient inducer of resistance to EBV infection than interferon γ. They also showed that in BJAB cells interferon α induced 2,5-oligoadenylate synthetase activity, whereas interferon γ increased the expression of HLA class I antigens.

In the present study the relative resistance/susceptibility to the anti-EBV effects of interferon were found to vary, not only with the host cell line, but were dependent also on the type of interferon used and the specific gene product affected.

Materials and Methods

Cell lines

Raji and Daudi are lymphoid cell lines derived from Burkitt's lymphoma tumors. Raji is a non-producer and Daudi is a low-level producer. The cell lines were maintained in RPMI-1640 medium supplemented with 10% FCS, 2 mM 1-glutamine and 100 μg/ml gentamicin in a humidified atmosphere with 5% CO_2. They were subcultured twice weekly to a concentration of 2×10^5/ml.

EBV preparation

P3HR-1 cells were cultured at a concentration of 2×10^5/ml for two weeks in RPMI-1640 containing 2% FCS at 34°C in a humidified atmosphere with 5% CO_2. Concentrated virus was prepared from the supernatant of the culture by centrifugation at $20\,000 \times g$ for 90 min in a Sorvall centrifuge. The pellets were resuspended in RPMI-1640 medium at 1/100 of the original volume and stored at −70°C.

Interferons

Recombinant interferons used in these experiments were kindly supplied by Dr. S. Chatterjee, University of Alabama at Birmingham, Birmingham, AL. Interferons α_2 and γ were obtained from Schering-Plough Corp., Bloomfield, NJ and interferon β from Cetas Corp., Emeryville, CA.

Interferon and infection assay

10^6 cells/tube were pretreated with 1–1000 U/ml of the different interferons and incubated at 37°C overnight. The interferon was washed off and the cells superinfected with EBV for 1 h at 37°C, washed and resuspended in 4 ml fresh RPMI medium. Following 2 days of incubation at 37°C, the cells were counted in each tube, washed 3 times with PBS, then spotted on slides and air dried.

Antibodies

Monoclonal antibodies against EA(D) and EA(R) were provided by Dr. Gary Pearson, Georgetown University, Washington, DC. Anti-EA(D) (R.3.1) is a mouse monoclonal antibody against the diffuse component of the early antigen complex. Anti-EA(R) (R.63.2) is directed against the restricted component of the EA− complex. Both antibodies were used at a dilution of 1:200 in the immunofluorescence assays. FITC-labeled goat anti-mouse IgG (Southern Biotechnology Associates, Birmingham, AL) at 1:20 was used as the second antibody.

Immunofluorescence

The cells were fixed for 10 min in acetone, washed in PBS and stained for immunofluorescence with the mouse monoclonal antibodies and FITC-labeled goat anti-mouse IgG. The slides were then counterstained with 0.1% Evan's blue for 5 min and the number of FITC-positive cells on each smear was determined using an American Optical Series 10 microscope with incident light fluorescence. 500 cells were counted in each smear.

Calculation of results

In order to combine results from several experiments, the numbers of EA-positive cells in interferon (IFN)-pretreated cultures were converted to percentages of the control EA expression by the following formula:

$$\% \text{ control} = \frac{\text{No. of EA+ cells IFN pretreated} \times 100}{\text{No. of EA+ cells untreated control}}$$

Both IFN-pretreated and -untreated cultures were infected in parallel with the same concentration of P3HR-1 EBV. The multiplicity of infection used in these experiments resulted in $1.0–1.5 \times 10^5$ EA+ cells in Raji or Daudi following infection of 10^6 cells. The geometric mean % control at each IFN concentration was calculated from 3–6 experiments. The dose of interferon required to inhibit EA expression by 50% (ED_{50}) was determined by regression analysis, using the power fit formula $y = ax^b$, where y = % control, x = units of IFN, a = intercept (value of y at 1 unit of IFN) and b = slope. The

correlation coefficient value (r) was >0.94 in each of the regression analyses. The average $\log_{10}$ SEM of the % control values was 0.085.

Results

As previously shown with human leukocyte interferon, EA-D was relatively resistant to interferon α_2 pretreatment of Raji cells and was relatively sensitive to the effects of interferon α on Daudi cells (Fig. 1). These curves, analyzed for the amount of interferon required to produce 50% inhibition of EA-D expression (ED_{50}), revealed that 10 U/ml were required for Daudi cells (Fig. 1B) and >1000 U/ml for Raji cells (Fig. 1A). Surprisingly, however, EA-R expression following interferon-α treatment was just the opposite in these two

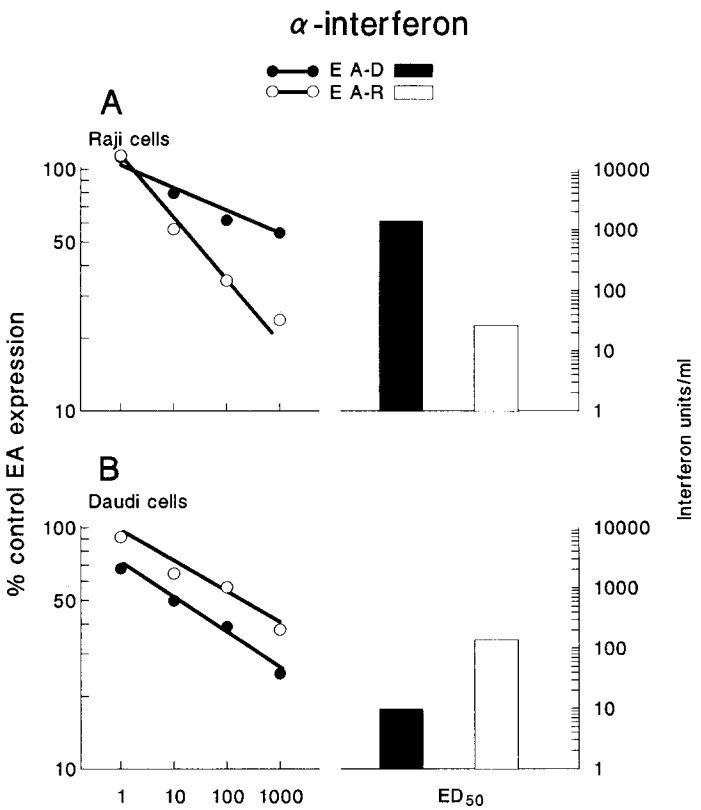

Fig. 1. Effects of α_2 IFN on EA expression in Raji (A) and Daudi (B) cells. Data on the left are expressed as % control EA expression in comparison to the number of cells expressing EA in parallel cultures not treated with interferon but infected with the same concentration of EBV. The % of control was calculated by the formula in the text. The lines represent the best fit curves ($r>0.98$) as determined by regression analysis. The columns on the right represent the ED_{50} for EA-D and EA-R calculated from the regression analyses. Each point represents mean values from 3–4 experiments; thus, each ED_{50} column is representative of 15–20 observations.

cell lines. EA-R expression was relatively sensitive to interferon-α pretreatment of Raji cells (ED_{50} = 27 U/ml) (Fig. 1A) and relatively resistant to interferon α in Daudi cells (ED_{50} = 143 U/ml) (Fig. 1B). It is perhaps noteworthy that the relative resistance of EA-R expression in Daudi cells is still 10-fold more sensitive than EA-D expression in Raji cells to pretreatment with interferon α.

Interferon β produced the most uniform anti-EBV EA activity on both cell lines against both gene products. 2–15 U/ml produced 50% inhibition of EA-D and EA-R in both cell lines (Fig. 2).

The differential pattern seen with EA-D and EA-R expression with interferon-α_2 pretreatment of Raji cells was reversed when the cells were pretreated with interferon γ. EA-D expression was reduced by 50% with 21 U/ml of γ interferon but almost 1800 U/ml were required for 50% inhibition of EA-R (Fig. 3A). A similar pattern of EA expression was found in Daudi cells following interferon-γ pretreatment. EA-D expression was decreased by 50% with 3 U/ml of interferon γ and >600 U/ml were required for 50% inhibition of EA-R (Fig. 3B). The cells were also evaluated for viability and total cell

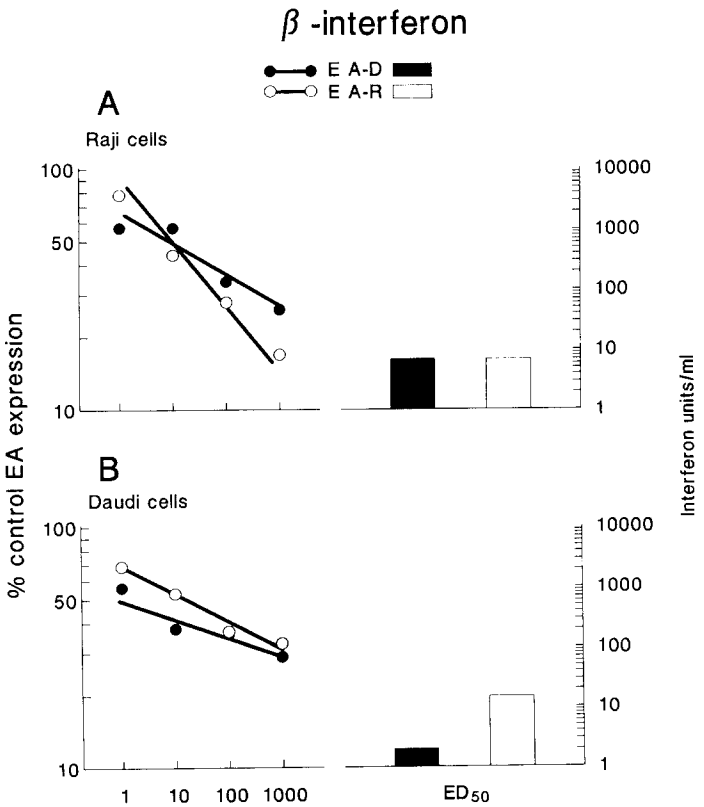

Fig. 2. Effects of β IFN on EA expression in Raji (A) and Daudi (B) cells. Data are expressed as in Fig. 1. Mean values from 4–5 experiments were used for each curve ($r \geq 0.95$). Calculated ED_{50} columns represent 20–25 observations.

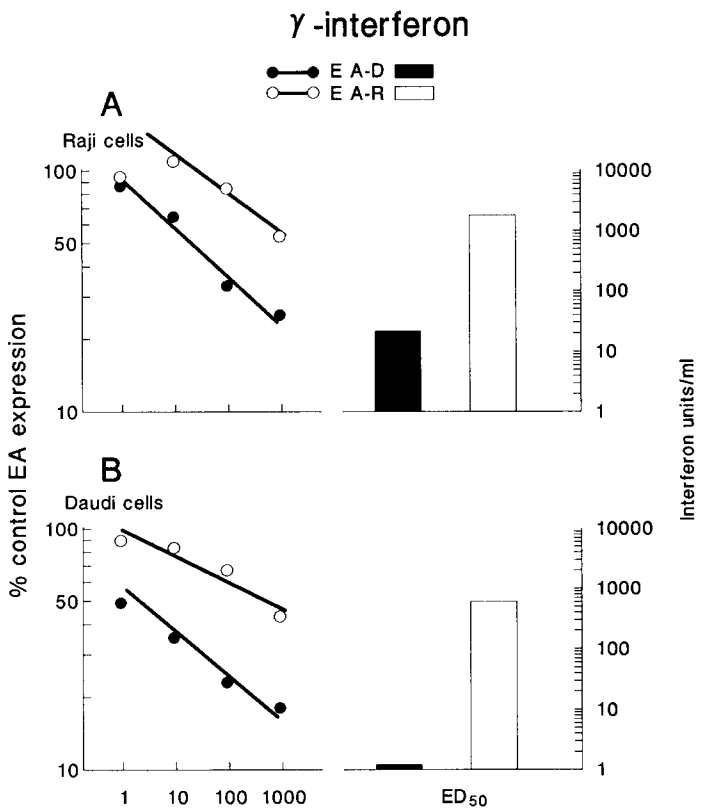

Fig. 3. Effects of γ IFN on EA expression in Raji (A) and Daudi (B) cells. Data are expressed as in Fig. 1. Mean values from 4–6 experiments were used for each curve ($r \geqslant 0.94$). The % control value for EA-R expression at 1 unit of IFN in Raji cells was excluded from the regression analysis. Calculated ED_{50} columns represent 15–25 observations.

number at the end of the two-day incubation period. No significant toxicity or growth inhibition was observed by pretreatment of either cell line with any of the recombinant interferons even at 1000 U/ml (data not shown).

Discussion

We have thus demonstrated a differential effect of human recombinant interferons α_2, β and γ on the expression of two specific early gene products of EBV. This differential activity varied with the cell line being superinfected but also with the affected gene product and the type of interferon used. We found no anti-proliferative nor toxic effects by pretreatment of the cells with recombinant interferons. The anti-proliferative activity requires continual presence of interferon during the course of the experiment (Adams et al., 1975b).

These data do not lend themselves to any unified simple model of susceptibility/resistance to the anti-EBV effects of interferon based on genetics of the treated cells. Neither receptor expression nor a genetic difference in regulation of the antiviral state explains the data observed. EA-D expression in Raji cells is resistant to interferon-α pretreatment. In contrast, EA-D expression in Daudi cells is very sensitive to interferon pretreatment. Based on these data alone one might speculate that Daudi cells express more interferon receptors than Raji cells. However, the relative sensitivity of EA-R expression by Raji cells and relative resistance of EA-R expression by Daudi cells following interferon-α pretreatment would seem to exclude this possibility. The results with interferons β and α also do not support receptor differences in the cell lines.

The genetic capacity of the cells to produce an antiviral state in response to interferons might play a role in the observed differences in EA expression following interferon pretreatment. The uniform susceptibility of both antigens in both cell lines to interferon β suggests no major difference in the capacity of either cell to produce an antiviral state following interferon-β pretreatment. In contrast, the differential expression of EA-D and EA-R following treatment with interferons α and γ suggests that multiple mechanisms may be involved in production of the antiviral state in response to these two interferons. Differential effects on viral transcription, translation or cell membrane alterations mediated by 2,5-oligo A, induction of protein kinase activity, (Joklik, 1985) or as yet unidentified mediators could be involved.

Differential effects of interferons on distinct viral gene products have been reported for one other herpes virus. Chatterjee and Burns (1990) have demonstrated a differential effect of interferons on expression of HSV-1 gene products. Pretreatment of human fibroblasts with interferon α or β, followed by HSV-1 infection, inhibited the expression of glycoproteins designated B and D. However, the expression of nucleocapsid proteins was unaffected (Chatterjee et al., 1985). Pretreatment of human neuroblastoma cells with interferon α likewise had little or no effect on the expression of HSV-1 nucleocapsid proteins but inhibited glycoproteins B and E and, to a lesser extent, D (Chatterjee and Burns, 1990).

The gene products evaluated in the present study, EA-D and EA-R, are encoded by two nonoverlapping gene segments near the center of the EBV genome (Farrell, 1989). EA-D is a 50-kDa early peptide that has sequence homology with ribonucleotide reductase (Goldschmidts et al., 1987). The precise molecular functions of these two gene products in viral replication are unknown. However, neither are integral viral proteins; both are early gene products that signal entry of the infected cell into productive virus replication. It has been assumed that these early gene products play a role in subsequent synthesis and/or assembly of later structural components of the virus. Alternatively, either or both could be involved in the inhibition of host-cell macromolecular synthesis that occurs concurrently with active synthesis of viral components (Gergely et al., 1971).

Conceivably, the differential effects of interferons α and γ on these two gene products could be a reflection of molecular events effected by the interferon and the gene product. For example, viral ribonucleotide reductase activity might abort an interferon activity based on inhibition of transcription or translation. Finally, a unique aspect of the current investigation deserves consideration. A major proportion of the observed EA expression (>80%) in Raji and Daudi cells following P3HR-1 EBV superinfection is a result of transactivation of resident viral genomes (Lidin et al., 1982). EA-D expression following P3HR-1 infection has been the major EBV-determined antigen studied in terms of transactivation by the Het gene product of P3HR-1 (Miller et al., 1984). However, Lieberman et al. (1986) have presented evidence that P3HR-1 can promiscuously transactivate gene products including those encoded by other viruses existing in a latent state in the targeted cells. We have found that EA-D and EA-R are equally transactivated in Raji and Daudi cells superinfected by P3HR-1 EBV (Lidin, unpublished observations). It is possible that the differential sensitivity of EA(D) and EA(R) to interferons α and γ could be due to their effects on transactivation.

As yet, we have no completely permissive system for replication of EBV. However, in terms of latency activation by induction (Hampar, 1979) or transactivation, EBV represents the best model available for detailed in vitro analyses. Thus, interferon regulation of EBV gene expression induced by transactivation is a unique system for evaluating discrete mechanisms involved in herpes virus latency activation and its inhibition.

Acknowledgements

Supported by V.A. Medical Research, contract No. 1-AI-82518 and grants PO1-CA-34968, RO1-CA-49609, RO1-CA-49609 and RO1-CA-38008 from the National Institutes of Health. Cell lines, monoclonal antibodies and advice on their use were kindly provided by Dr. Gary Pearson, Georgetown University, Washington, DC. We thank Dr. Subhendra Chatterjee for providing recombinant interferons and his helpful review of the manuscript, Joyce Watkins and Gwen Marshall for expert technical assistance and Jean Williams for preparation of the manuscript through its revisions.

References

Adams, A., Lidin, B., Strander, H. and Cantell, K. (1975a) Spontaneous interferon production and Epstein-Barr virus antigen expression in human lymphoid cell lines. J. Gen. Virol. 28, 207–217.

Adams, A., Strander, H. and Cantell, K. (1975b) Sensitivity of the Epstein-Barr virus and virus transformed human lymphoid cell lines to interferon. J. Gen. Virol. 28, 219–223.

Adams, A. (1979) The state of the virus genome in transformed cells and its relationship to host cell DNA. In: M.A. Epstein and B.G. Achong (Eds.), The Epstein-Barr Virus, Springer Verlag, New York, pp. 156–178.

Cantell, K. and Phyälä, L. (1973) Circulating interferon in rabbits after administration of human interferon by different routes. J. Gen. Virol. 20, 97–104.

Chatterjee, S., Hunter, E. and Whitley, R. (1985) Effect of cloned human interferons on protein synthesis and morphogenesis of Herpes Simplex virus. J. Virol. 56, 419–425.

Chatterjee, S. and Burns, P. (1990) Expression of Herpes Simplex virus Type 1 glycoproteins in interferon-treated human neuroblastoma cells. J. Virol. 64, 5909–5913.

Farrell, P. (1989) Epstein-Barr virus genome, In: G. Klein (Ed.), Advances in Viral Oncology, Raven Press, New York, pp. 103–127.

Gergely, L., Klein, G. and Ernberg, I. (1971) Effect of EBV-induced early antigens on host-cell macromolecular synthesis studied by combined immunofluorescence and radioautography. Virology 45, 22–29.

Goldschmidts, W., Luka, J. and Pearson, G.R. (1987) A restricted component of the Epstein-Barr virus (EBV) early antigen (EA) complex is structurally related to ribonucleotide reductase. Virology 157, 220–226.

Groopman, J.E., Sullivan, J.L., Mulder, C., Ginsburg, D., Orkin, S.H., O'Hara, C.J., Falchuk, K., Wong-Stahl, F. and Gallo, R.C. (1986): Pathogenesis of B-cell lymphoma in patients with AIDS. Blood 67, 612–615.

Hampar, B. (1979) Activation of the viral genome in vitro. In: M.A. Epstein and B.G. Achong (Eds.), The Epstein-Barr Virus, Springer Verlag, New York, pp. 283–296.

Hanto, D.W., Frizzera, G., Purtilo, D.T., Sakamoto, K., Sullivan, J.L., Saemundson, A.K., Klein, G., Simmons, R.L. and Najarian, J.S. (1981) Clinical spectrum of lymphoproliferative disorders in renal transplant recipients and evidence for the role of the Epstein-Barr virus. Cancer Res. 4, 4253–4261.

Henle, G., Henle, W. and Diehl, V. (1968) Relation of Burkitt's tumor-associated herpes-type virus to infectious mononucleosis. Proc. Natl. Acad. Sci. USA 59, 94–101.

Joklik, W.K. (1985) Interferons. In: (B.N. Fields et al. (Eds.), Fields Virology, Raven Press, New York, pp. 281–300.

Klein, G. and Dombos, L. (1973) Relationship between the sensitivity of EBV carrying lymphoblastoid lines to superinfection and the inducibility of the resident viral genome. Int. J. Cancer 11, 327–337.

Kure, S., Tada, K., Wada, J. and Yoshie, O. (1986) Inhibition of Epstein-Barr virus infection in vitro by recombinant human interferons α and γ. Virus Res. 5, 377–390.

Lidin, B. and Lamon, E.W. (1982) Antiviral effects of interferon on a somatic cell hybrid between two Burkitt's lymphoma cell lines of different interferon sensitivities. Infect. Immun. 36, 847–849.

Lidin, B., Lamon, E.W., Cloud, G. and Soong, S.J. (1982) Complementation between infecting Epstein-Barr virus and intrinsic viral genomes. Intervirology 18, 66–75.

Lieberman, P.M., O'Hare, P., Hayward, G.S. and Hayward, S.D. (1986) Promiscuous transactivation of gene expression by an Epstein-Barr virus-encoded early nuclear protein. J. Virol. 60, 140–148.

Miller, G., Rabson, M. and Huston, L. (1984) Epstein-Barr virus with heterogeneous DNA disrupts latency. J. Virol. 50, 174–182.

Nonoyama, M., Huang, C.H., Pagano, J.S., Klein, G. and Singh, S. (1973) DNA of Epstein-Barr virus detected in tissue of Burkitt's lymphoma and nasopharyngeal carcinoma. Proc. Natl. Acad. Sci. USA 70, 3265–3268.

Purtilo, D.T., Sakamoto, K., Barnabai, V., Seeley, J., Bechtold, T., Rogers, G., Yetz, J. and Harada, S. (1982) Epstein-Barr virus induced diseases in gays with the X-linked lymphoproliferative syndrome (XLP): updates on studies of the registry. Am. J. Med. 73, 49–56.

Sullivan, J.L., Byron, K.S., Brewster, F.E. and Purtilo, D.T. (1980) Deficient natural killer cell activity in X-linked lymphoproliferative syndrome. Science 210, 543–545.

Takada, K., Shimizu, N., Sakuma, S. and Ono, Y. (1986) Transactivation of the latent Epstein-Barr virus (EBV) genome after transfection of the EBV DNA fragment. J. Virol. 57, 1016–1022.

Vilcek, J., Gresser, I. and Merigan, T.C. (1979) Regulatory functions of Interferons, New York Academy of Sciences, New York.

Zur Hausen, H., Schulte-Holthausen, H., Klein, G., Henle, W., Henle, G., Clifford, P. and Santessen, L. (1970) EBV DNA in biopsies of Burkitt's lymphoma and anaplastic carcinomas of the nasopharynx. Nature (London) 228, 1056–1058.

Zur Hausen, H., Diehl, V., Wolf, H., Schulte-Holthausen, H. and Schneider, U. (1973) Occurrence of Epstein-Barr virus genomes in human lymphoblastoid cell lines. Nature New Biol. 237, 189–190.