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Research Article

Infection by herpesviruses can result in profound immunosuppressive or immunomodulatory effects. However, no significant information is available on the effect of such infections on the production of immunoregulatory cytokines. We studied the kinetics of production of two monocyte-derived cytokines, interleukin 6 (IL-6) and tumor necrosis factor-alpha (TNF alpha), induced by Epstein-Barr virus (EBV) and herpes simplex virus type 1 (HSV-1) in peripheral blood mononuclear cell cultures and in fractionated cell populations. We observed that, when compared to HSV-1, EBV is a stronger inducer of IL-6. In EBV-infected cultures, IL-6 protein was detected at day 1 postinfection and gradually increased with time. In contrast, lower amounts of IL-6 were detected 5 d postinfection in HSV-1-infected cultures. HSV-1-infected cultures secreted significant amounts of TNF alpha protein after 5 d of culture and reached a maximal level of production at day 7, whereas EBV inhibited TNF alpha production. In fractionated cell populations, monocytic cells were found to be the main source of IL-6 synthesis after EBV or HSV-1 infection. However, TNF alpha synthesis in HSV-1-infected cultures was from both B and monocytic cells. By using the polymerase chain reaction technique we show that, after infection by these two herpesviruses, differences in cytokine gene products are also observed at the transcriptional level. These observations demonstrate that EBV and HSV-1 exert differential effects on IL-6 [...]

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Infection of Peripheral Blood Mononuclear Cells by Herpes Simplex and Epstein-Barr Viruses

Differential Induction of Interleukin 6 and Tumor Necrosis Factor- α

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Abstract

Infection by herpesviruses can result in profound immunosuppressive or immunomodulatory effects. However, no significant information is available on the effect of such infections on the production of immunoregulatory cytokines. We studied the kinetics of production of two monocyte-derived cytokines, interleukin 6 (IL-6) and tumor necrosis factor- α (TNF α), induced by Epstein-Barr virus (EBV) and herpes simplex virus type 1 (HSV-1) in peripheral blood mononuclear cell cultures and in fractionated cell populations. We observed that, when compared to HSV-1, EBV is a stronger inducer of IL-6. In EBV-infected cultures, IL-6 protein was detected at day 1 postinfection and gradually increased with time. In contrast, lower amounts of IL-6 were detected 5 d postinfection in HSV-1-infected cultures. HSV-1-infected cultures secreted significant amounts of TNF α protein after 5 d of culture and reached a maximal level of production at day 7, whereas EBV inhibited TNF α production. In fractionated cell populations, monocytic cells were found to be the main source of IL-6 synthesis after EBV or HSV-1 infection. However, TNF α synthesis in HSV-1-infected cultures was from both B and monocytic cells. By using the polymerase chain reaction technique we show that, after infection by these two herpesviruses, differences in cytokine gene products are also observed at the transcriptional level. These observations demonstrate that EBV and HSV-1 exert differential effects on IL-6 and TNF α gene transcription and on the resulting protein secretion in human mononuclear blood cells. (*J. Clin. Invest.* 1992. 89:1849-1856.) Key words: cytokines • herpesviruses • immunoregulation • viral infection

Introduction

Herpesviruses are ubiquitous and highly pathogenic and can produce acute, chronic, latent, or recurring infections in their natural hosts. Epstein-Barr virus (EBV) is known to infect and transform human B lymphocytes in vitro. Herpes simplex

virus type 1 (HSV-1)¹ also interacts intimately with the immune system by infecting lymphoid cells. Viral reactivation is common to both these viruses and can result in severe infections, especially in immunodeficient hosts (1, 2).

The immune system plays a key role in the control/surveillance of virus-infected cells. In this regard, T cell-derived cytokines such as interleukin 2 (IL-2) (3-5) and interferon- γ (IFN γ) (3, 4, 6) are well known for their cellular regulatory roles in host defense mechanisms. Furthermore, interleukin 6 (IL-6) and tumor necrosis factor- α (TNF α), which are produced mainly by monocytic cells (7-9), are known to act on a variety of target cells. For example, IL-6 is involved in the induction of B cell differentiation, induction of IL-2 and IL-2 receptor expression, and in induction of proliferation and differentiation of T cells (for review, see reference 10). TNF α also exerts diverse physiological functions such as increasing macrophage cytotoxicity (11, 12), mediation of natural cellular cytotoxicity (13) and playing regulatory roles in T and B cell activation and maturation (14, 15). In the HSV-1 system, little is known about the regulatory roles played by cytokines. Previous reports suggest that exogenous IL-2 and IFN γ protected neonatal mice from a lethal HSV infection (16, 17). Furthermore, it has been shown that EBV-infected human peripheral blood mononuclear cells (PBMC) can produce IL-2 and IFN γ (18) and that these cytokines play a crucial role in the resistance of EBV infection (19, 20). However, in both systems, little is known about the role of IL-6 and TNF α in the immunosurveillance of infected cells.

We have shown in a previous report that EBV can interact with monocytic cell function by reducing the levels of TNF α transcription and protein synthesis (21). To learn more about the key role played by the monocytic cells and the monokines they secrete during viral infections, we compared the effects of two herpesviruses, EBV and HSV-1, on the transcription and release of IL-6 and TNF α by PBMC. We observed that EBV is a better inducer of IL-6 than HSV-1. In contradistinction to EBV, HSV-1 was revealed to be an inducer of TNF α activity. The different monokine RNA levels detected in virus-infected cells using polymerase chain reaction (PCR) suggest that EBV and HSV-1 can exert their effects at the transcriptional level.

Methods

Cell preparations. PBMC from EBV and HSV-1 antibody-negative (seronegative) and antibody-positive (seropositive) healthy donors were

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1. **Abbreviations used in this paper:** EBNA, Epstein-Barr virus nuclear antigen; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HSV-1, herpes simplex virus type 1; PCR, polymerase chain reaction; TNF α , tumor necrosis factor- α .

obtained by centrifugation of heparinized blood over Ficoll-Hypaque gradients (Pharmacia, Uppsala, Sweden). The EBV-seropositive or -seronegative status of these donors was defined by the presence or absence, respectively, of antibodies to EBV capsid antigen and to EBV nuclear antigen (EBNA) by using immunofluorescence assays as described earlier (22, 23). Seropositivity for HSV-1 was confirmed by standard complement fixation test. Fractionated/enriched cell populations were obtained by incubating PBMC at least once with known specific antibodies and rabbit complement as described (24). Antibodies used were anti-human T cells (OKT3, OKT11), anti-human B cells (OKB7), and anti-human monocytes (OKM1, OKM14) (Ortho Diagnostic Systems, Raritan, NJ). The purity of fractionated mononuclear cell populations used was evaluated by flow cytometry after an immunofluorescence test using specific antibodies described above and was as follows: 94–97% for T cells, 87–93% for B cells, and 88–92% for monocytes.

Viruses and mitogen stimulation. The procedures followed for preparation and titration of cell-free EBV (strain B95-8) and HSV-1 (strain McIntyre) were as described previously (22–23, 25–27). Briefly, fractionated mononuclear cells and PBMC (1×10^6 cells) were infected with 200 μ l of EBV preparation with a titer of 2×10^5 EBNA-inducing U/ml or with HSV-1 preparation at a multiplicity of infection of 50 plaque-forming units (PFU) per cell. After 1 h of incubation at 37°C, 5% CO₂ in air, and humid atmosphere, 0.8 ml of culture medium was added and resuspended cells were incubated. Viral preparations and culture medium tested for the presence of endotoxin by the limulus amoebocyte assay (Sigma Chemical Co., St. Louis, MO) were found to contain < 20 pg/ml of contaminating endotoxin. The viral preparations used contained undetectable levels of IL-1, IL-2, IL-3, IL-4, IL-6, IL-8, and TNF, as evaluated by using enzyme-linked immunosorbent assay (ELISA; see below). PBMC (1×10^6 cells) stimulated with lipopolysaccharides (LPS) (1.0 μ g/ml) were used as positive control. Cell cultures (1×10^6 cells/ml) treated with EBV, HSV-1, and LPS were grown in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 50 U/ml penicillin, 50 μ g/ml streptomycin, 30 μ g/ml gentamycin, and 10 mM HEPES buffer. Cell-free supernatants from each infected and uninfected PBMC cultures were collected on different days of culture (days 1, 3, 5, and 7) and stored at -70°C until used for cytokine determinations.

Assay for cytokine activities. Supernatants of virus-infected and LPS-treated cultures as well as those of uninfected control cultures were collected on different days for measurement of IL-6 and TNF α activities. Cytokines were quantitated using ELISA kits, according to the manufacturer's technical guidelines (R & D Systems, Inc., Minneapolis, MN).

Cytokine mRNA analysis. Cells were lysed at 8 h posttreatment and total RNA was isolated using a modified guanidium isothiocyanate procedure (28). Cytokine mRNA levels were analyzed by using a reverse transcriptase-PCR procedure as described (29). PCR products were analyzed on a 6.0% denaturing PAGE and autoradiographed overnight at -70°C using Cronex film (DuPont Co., Wilmington, DE). The following primers were used: TNF α primer A located at 2199–2175 (5'-CCCTCAAGCTGAGGGCAGCTCCAG-3') and primer B located at 2826–2801 (5'-GGGCAATGATCCCAAAGTAGACCTG-3') of the fourth TNF α exon; IL-6 primer A located at 4144–4124 (5'-CAAAGAATCTAGATGCAATAA-3') and IL-6 primer B located at 4320–4299 (5'-GCCCATTAACAACAACAATCTG-3') in the IL-6 cDNA. The glyceraldehyde 3-phosphate dehydrogenase (GAPDH) product was amplified using primer A (5'-GGTACCTCTTCCGACCCC-3') located at 389–372 and primer B (5'-GTTTCAACAGTACCTACTGG-3') located at 566–547 derived from a full-length GAPDH cDNA (30). The intensity of the PCR products was determined by laser densitometry. For each sample the amount of TNF α and IL-6 mRNAs was quantitated relatively to their respective level of GAPDH mRNA. The given ratio was compared to the one obtained (i.e., the internal basal mRNA control) (30) in unstimulated cells using the following formula: (OD of cytokine mRNA transcripts of the sample/OD of GAPDH transcripts of the sample)/(OD of cytokine mRNA

transcripts of unstimulated cells/OD of GAPDH mRNA transcripts of unstimulated cells.) = relative mRNA level. Constant amplification in reaction tubes was assessed by coamplifying exogenous DNA (SV₂CAT) in some samples. Furthermore, all reactions were carried out in conditions previously described (29) in which amplification was linear.

Results

To determine the cytokine inducing potential of both HSV-1 and EBV, PBMC from seronegative and seropositive donors were infected in parallel with each virus and their culture supernatants were assayed on different days postinfection for IL-6 and TNF α activities. It was thus found that 50 PFU/cell of HSV-1 and 2×10^5 EBNA-inducing U/ml of EBV represent optimal doses for cytokine inductions (Table I).

IL-6 synthesis by PBMC. The results given in Fig. 1 indicate that EBV induces IL-6 protein synthesis. Production of IL-6 was readily detected in PBMC cultures from seropositive donors, 24 h after EBV infection. IL-6 synthesis increased steadily and reached a maximal level 7 d postinfection. In contrast to EBV, HSV-1 did not induce significant levels of IL-6 protein before day 5 postinfection, in that the amounts of IL-6 detected during this period were below the background level. Comparable results were obtained with PBMC from seronegative donors (Fig. 2). In fact, IL-6 production was detected at day 3 postinfection and reached a maximal level by day 7. In HSV-1-infected PBMC cultures, IL-6 synthesis was detected at day 3 but decreased after 7 d postinfection. However, even if IL-6 and TNF α production patterns were similar, a higher level of protein was detected in PBMC cultures from seropositive donors than in PBMC culture from seronegative donors.

Table I. Induction of Cytokine Synthesis by HSV-1 and EBV in PBMC at Different Multiplicities of Infection

Virus multiplicity of infection	5 d postinfection	
	IL-6	TNF α
	pg/ml	
HSV-1 (PFU/cell)		
12.5	840	49
25	890	58
50	3,150	115
100	1,300	110
200	620	65
EBV (EBNA-inducing U $\times 10^5$)		
0.5	820	—
1.0	1,600	—
2.0	8,100	—
5.0	3,640	—

Results given here are for day 5 postinfection and are expressed in picograms per milliliter in which background levels from unstimulated cell controls were subtracted. These results are means of two separate determinations and were obtained using PBMC of a seropositive healthy donor. Although not shown here, results of samples tested on day 7 postinfection also indicated that 50 PFU/ml for HSV-1 and 2×10^5 EBNA-inducing U/ml for EBV represent optimal doses for cytokine induction by these two herpesviruses. (Details on cytokine kinetics are given in Figs. 1–4.) Dashes indicate values below the unstimulated (control) level.

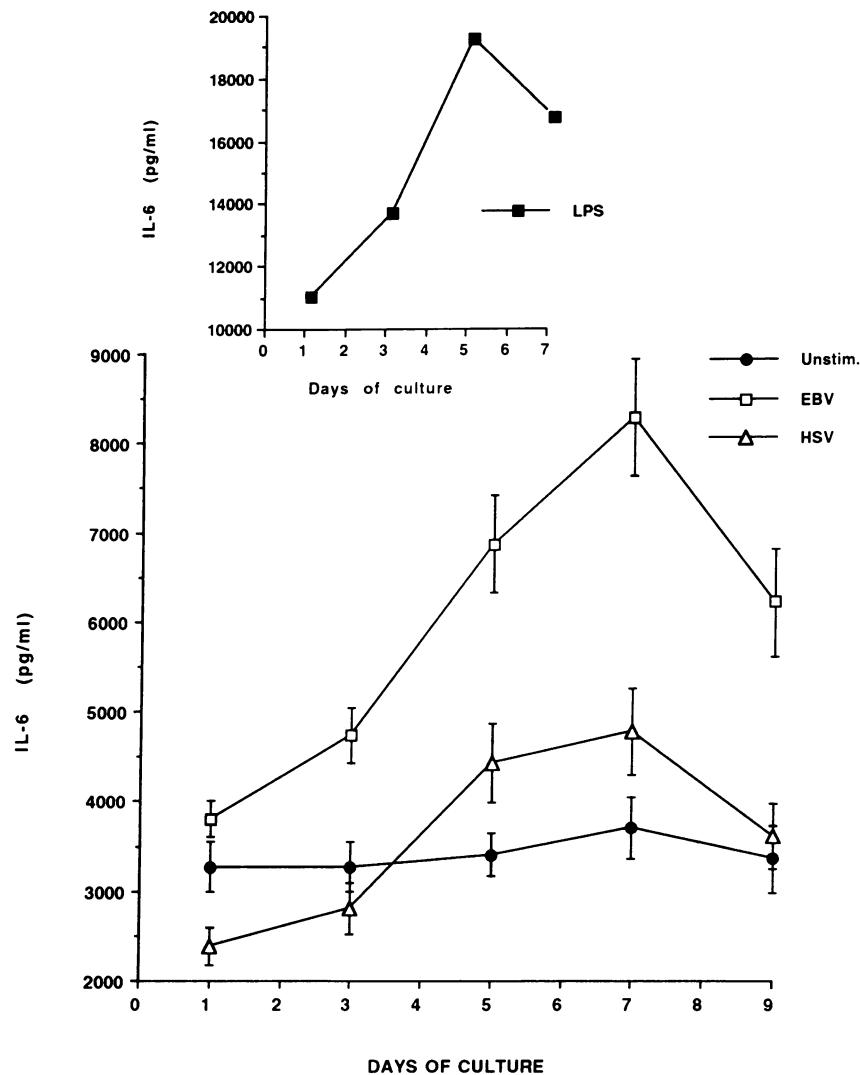


Figure 1. Kinetics of IL-6 activity in EBV- and HSV-1-infected PBMC culture supernatants from seropositive donors. IL-6 production was measured by ELISA kits (see Methods). Upper panel shows the LPS-induced cytokine activity used as positive control. Detection limit was 3.5 pg/ml. Viral infection was confirmed by immunofluorescence on cells harvested on day 3 post-infection. Cellular viability was > 90% in all cell cultures. These data represent means of three separate experiments \pm SD. Viral preparations pretreated with human sera containing virus-neutralizing antibodies were unable to induce cytokine production (not shown).

TNF α synthesis by PBMC. During the first 3 d postinfection, the amounts of TNF α protein detected in culture supernatants of EBV-infected as well as of HSV-1-infected PBMC from seropositive donors were not higher than the level of spontaneous release by unstimulated cells (Fig. 3). However, in HSV-1-infected cultures, TNF α production was found increased at day 5 postinfection and reached a maximal level after 7 d of culture. In supernatants from EBV-infected PBMC cultures, the amounts of TNF α detected at different times postinfection were always less than in those from unstimulated cultures, regardless of the viral antibody status of the donor, suggesting that EBV suppressed TNF α protein secretion (Fig. 4).

Cytokine production by fractionated cell populations. In an attempt to determine whether monocytic cells are directly stimulated by these viruses to produce cytokines or if another cell population is involved in such a cytokine synthesis, we performed the same sets of experiments, as above, using fractionated cell populations. As shown in Table II, monocytic cells were found to be the major source of IL-6, although B cells did also produce significant amounts of IL-6 protein. Furthermore, as observed in PBMC culture supernatants, EBV was found to be a stronger IL-6 inducer than HSV-1. As expected, no IL-6 protein was detected in culture supernatants of enriched T cell

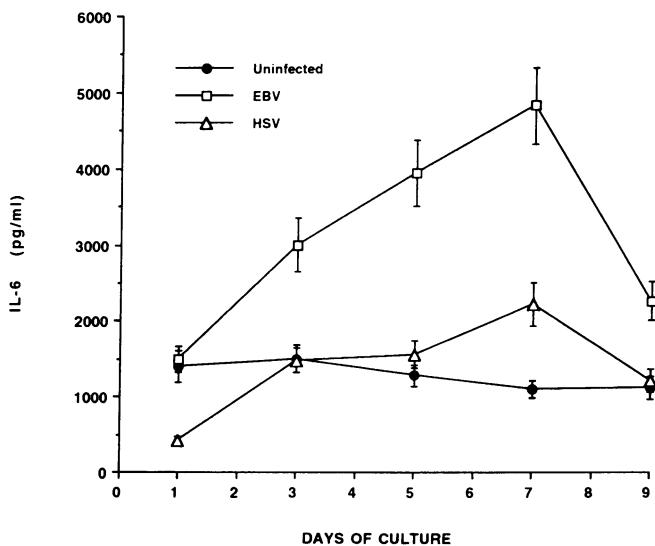


Figure 2. Kinetics of IL-6 activity in EBV- and HSV-1-infected PBMC from seronegative donors. (See legend to Fig. 1 for additional experimental details.) These data represent means of three separate experiments \pm SD.

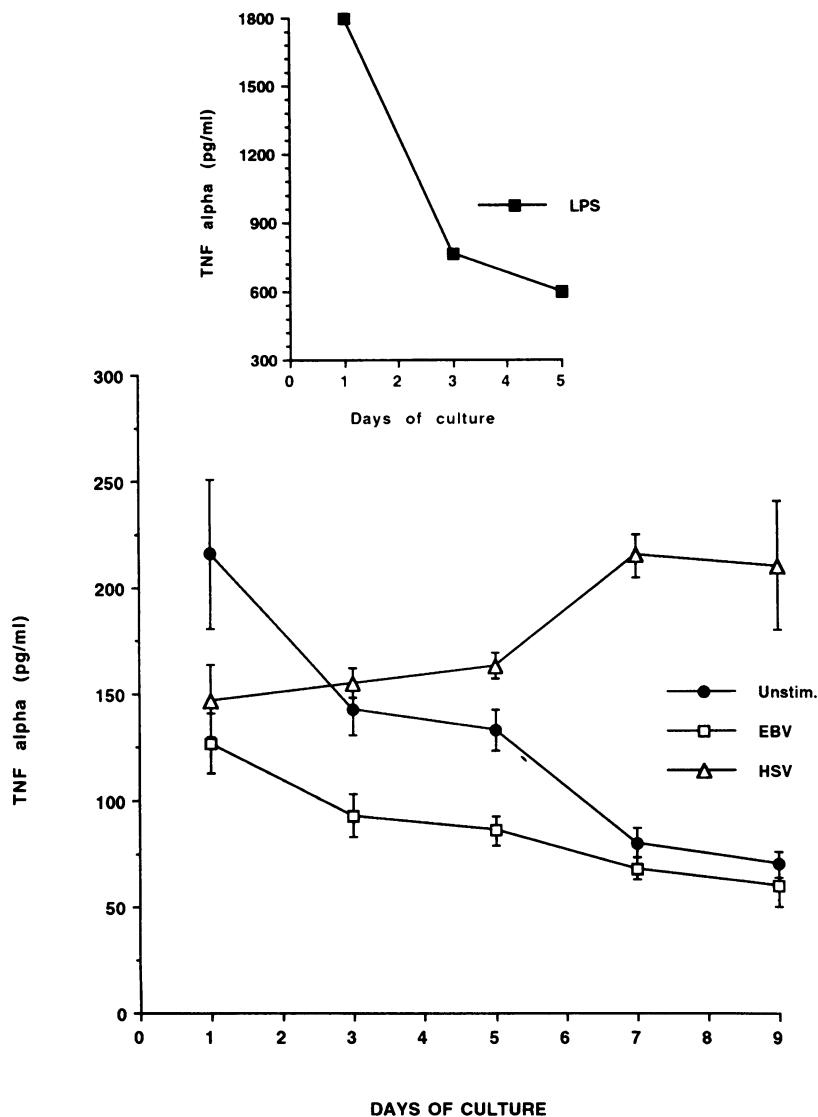


Figure 3. Kinetics of TNF α release in EBV- and HSV-1-infected PBMC culture supernatants from seropositive donors. TNF α production was measured by ELISA kits (see Methods). Upper panel shows the LPS-induced cytokine activity used as positive control. Detection limit was 4.8 pg/ml. These data represent mean of three separate experiments \pm SD. Viral preparations pretreated with neutralizing antibodies were unable to induce cytokine production.

populations. These results suggest that both viruses can directly act on monocytic cells to induce IL-6 production. On the other hand, TNF α was also produced by other than monocytic cells after HSV-1 infection. In fact, TNF α protein levels in culture supernatants of enriched B cells were comparable to those of monocytic cell populations (Table III). No TNF α protein was detected in EBV-treated, fractionated cell populations.

Effects of inactivated viruses on cytokine production. To determine the role of inactivated virus particles in cytokine induction, additional experiments were performed. EBV and HSV-1 preparations were inactivated by treatment with ultraviolet light (UV) before their use in the infection procedure; culture supernatants were harvested at different times posttreatment and assayed for the presence of IL-6 and TNF α (Table IV). The results obtained show that UV irradiation of EBV did not affect the IL-6-inducing ability of the virus particle, suggesting a role for a viral structural (envelope) protein in this IL-6 induction. On the other hand, IL-6 protein levels were increased by the use of UV-irradiated HSV-1. This suggests that infectious HSV-1 might suppress IL-6 synthesis. However, UV irradiation of HSV-1 did not affect the TNF α protein release suggesting that HSV-1 infectivity/replication is not re-

quired for TNF α induction. This contrast with EBV-treated cultures, where the TNF α protein levels were not affected by the use of UV-irradiated particles whereas infectious EBV had a down-regulatory effect on this cytokine's synthesis.

Analysis of mRNAs for IL-6 and TNF α . To extend our study at the molecular level, we evaluated the effects of viral infection on cytokine gene expression using the PCR technique. The steady-state levels of IL-6 and TNF α mRNA were examined in unstimulated, LPS-, EBV-, and HSV-1-infected PBMC at 8 h posttreatment. As shown in Fig. 5 (left), after amplification (201 basepair fragment), IL-6 transcripts were detected in LPS-treated cells (lane 2), as well as in EBV- (lane 3) or HSV-1-infected cells (lane 4). In the same total RNA extracts, TNF α transcripts (Fig. 5, right) were also detected (lanes 3, 4, and 5, respectively). Total length of the amplified sequence from the fourth TNF α exon is a 412 basepair fragment. To quantitate the IL-6 and TNF α mRNA levels induced in infected PBMC, the autoradiographs were examined by laser densitometry and the fold increase in cytokine's mRNA in each sample was calculated relative to the basal levels of GAPDH RNA in the sample. As shown in Fig. 6, IL-6 transcripts were weakly induced in HSV-1-treated PBMC com-

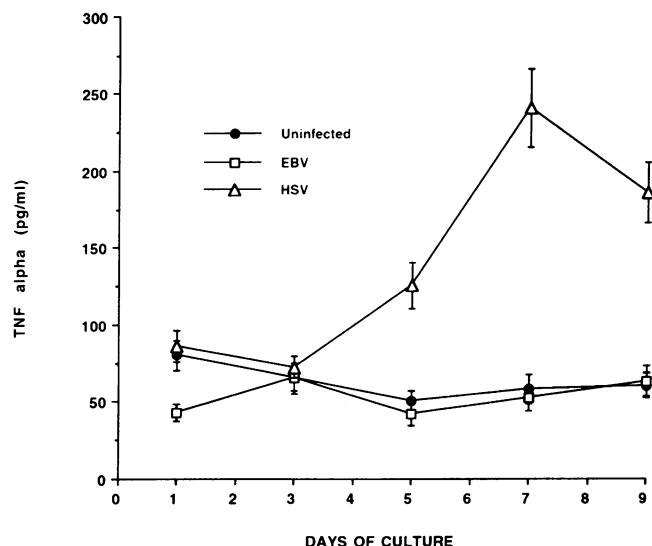


Figure 4. Kinetics of TNF α synthesis by EBV- and HSV-1-infected PBMC from seronegative donors. (See legend to Fig. 1 for experimental details.) These data represent the mean of three separate experiments \pm SD.

pared to those in EBV-infected cultures. On the other hand, IL-6 mRNA level was found increased threefold in EBV-infected cells, as compared to untreated cells (controls). In contrast, TNF α mRNA levels were increased 3.8-fold in HSV-1-infected cells but not in EBV-infected cells.

Table II. Kinetics of Production of IL-6 by Fractionated/Enriched Cell Populations Treated with HSV-1 and EBV

Cell populations	Days postinfection				
	1	3	5	7	9
	pg/ml				
HSV-1 treated					
B cells	ND	6,100	6,150	5,000	3,280
T cells	ND	—	—	—	—
Monocytes	ND	—	5,100	12,200	6,140
EBV treated					
B cells	—	9,200	8,100	4,230	3,900
T cells	—	—	—	—	—
Monocytes	10,100	13,544	21,240	23,130	8,500

Values shown are means from two separate experiments, each carried out in triplicate, where background levels from unstimulated cells (controls) were subtracted. Values between samples of each enriched population differed by < 10%. Cells were infected with HSV-1 at a concentration of 50 PFU/cell and with EBV at a concentration of 2×10^5 EBNA-inducing U/ml as described above (see Table I). ND, not done. Dashes indicate values below the unstimulated (control) level. Cells from two different (seropositive) donors were used in parallel for these IL-6 determinations. Cell samples examined on days 3 and 7 by immunofluorescence for HSV-1 antigens gave positive results. For EBV, with the exception of B cell culture samples, which were found positive by immunofluorescence for the EBNA, T and monocytic cells were found negative when tested for both EBNA and the EBV early antigen (results not shown).

Table III. Kinetics of Production of TNF α by Fractionated/Enriched Cell Populations Treated with HSV-1 and EBV

Cell populations	Days postinfection				
	1	3	5	7	9
	pg/ml				
HSV-1 treated					
B cells	ND	150	165	180	110
T cells	ND	—	—	ND	ND
Monocytes	ND	102	160	195	50
EBV treated					
B cells	—	—	—	ND	ND
T cells	—	—	—	ND	ND
Monocytes	—	—	—	ND	ND

Experimental details are the same as described in Table II. ND, not done. Dashes indicate values below the unstimulated (control) level.

Discussion

This study shows that the two herpesviruses investigated, EBV and HSV-1, can differentially regulate the syntheses of two cytokines of monocytic origin (i.e., IL-6 and TNF α), irrespective of the serological status of the individual. This is exemplified by the fact that, in contrast to HSV-1, EBV revealed to be a more powerful inducer of IL-6 protein. In PBMC cultures, IL-6 was found to increase up to day 7 posttreatment. This IL-6 induction seems to be associated with an early event of virus-cell interaction in that IL-6 production by B cells peaked 3–5 d postinfection. Furthermore, we did not detect significant amounts of IL-6 protein in culture supernatants of EBV-transformed B cell cultures (data not shown).

Previous studies have shown that recombinant human IL-6 can promote the proliferation of EBV-infected human B cells

Table IV. Effect of Inactivated Viruses on IL-6 and TNF α Synthesis by PBMC

Cell treatment	IL-6	TNF α
	pg/ml	
Unstimulated cells	3,250	125
Infectious EBV	6,500	90
UV-irradiated EBV	6,150	120
Heat-inactivated EBV	3,000	115
Infectious HSV-1	3,600	160
UV-irradiated HSV-1	4,400	175
Heat-inactivated HSV-1	3,200	95

Culture supernatants were harvested at day 5 posttreatment and assayed for cytokine activities. Virus inactivation was carried out using heat (56°C/60 min) or UV irradiation (265 nm/60 min) and was confirmed by the fact that heat- or UV-treated virus preparations (in contrast to untreated control virus samples) were unable to infect susceptible targets (i.e., BJA-B cells for EBV and Vero cells for HSV-1) as tested by standard procedures (22, 23, 26). Data represent means from two separate experiments, each carried out in triplicate, using cells from the same seropositive donor. Values among triplicate samples varied by < 10%.

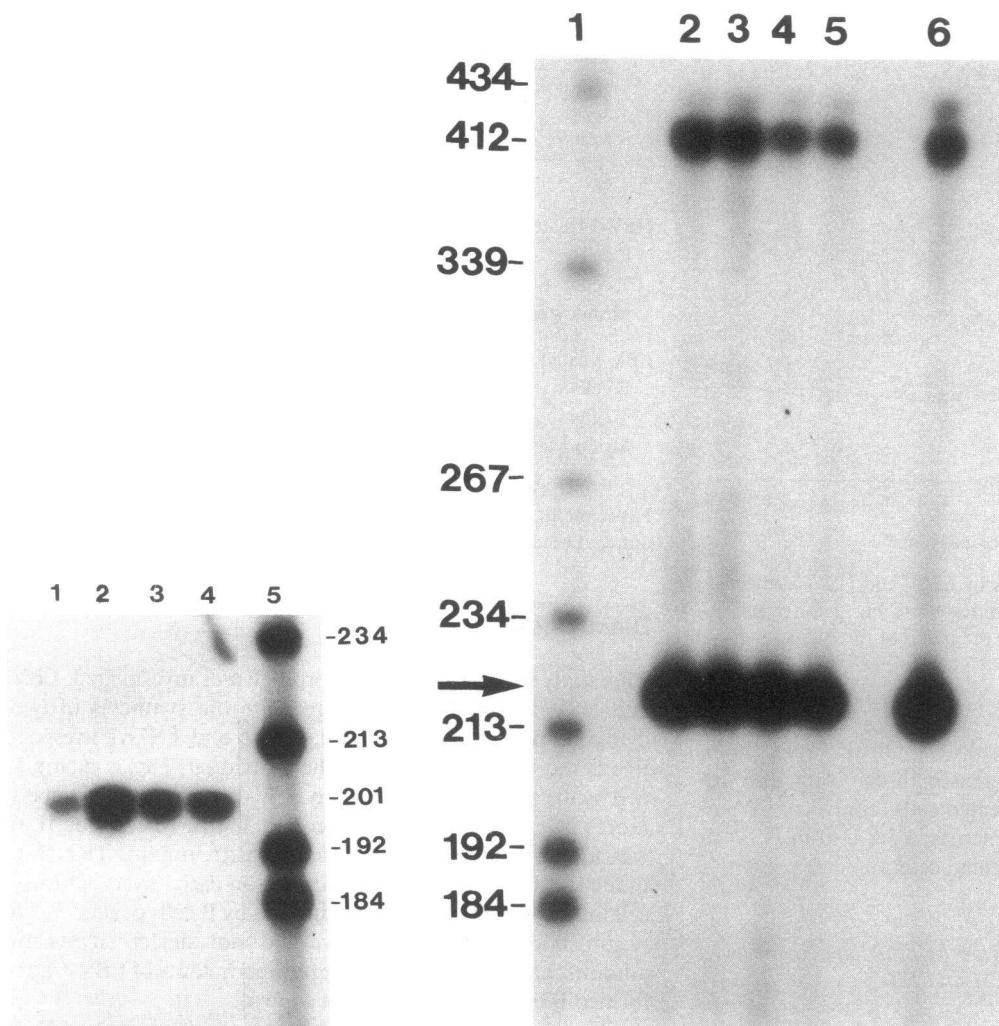


Figure 5. Cytokine mRNA levels quantitated by PCR. (*Left*), IL-6 transcripts of treated PBMC: lane 1, unstimulated PBMC; lane 2, LPS-treated PBMC; lane 3, EBV-infected PBMC; lane 4, HSV-1-infected PBMC; lane 5, markers (pAT153XHaeIII). (*Right*) TNF α transcripts of treated PBMC: lane 1, markers; lane 2, unstimulated PBMC; lane 3, LPS-treated PBMC; lane 4, EBV-infected PBMC; lane 5, HSV-1-infected PBMC; lane 6, Sendai virus-induced TNF α in U937 cells (positive control). TNF α and SV₂CAT templates were coamplified by PCR for each sample. The size of PCR products for SV₂CAT DNA (220 bp) is indicated by an arrow.

(31, 32), suggesting that IL-6 might indeed be used as a paracrine/autocrine growth factor by these cells (33). Another recent study indicated that expression of an exogenous IL-6 gene in EBV-transformed B cells confers growth advantage and in vivo tumorigenicity (34). The increased amounts of IL-6 protein that we have measured in the culture supernatants of EBV-infected PBMC support these observations.

Interestingly, HSV-1 appears to have a weak IL-6 inducing ability which is detectable only 5 d postinfection. Our results also suggest that IL-6 production is delayed in cell cultures treated with infectious HSV-1 compared with inactivated virus.

The fact that the production of TNF α was different in PBMC cultures infected by each virus is also very interesting. In EBV-infected PBMC cultures, the detectable level of TNF α protein was below that of uninfected cultures (controls), even when examined 7 d postinfection. TNF α is known to have antiviral activity against certain DNA and RNA viruses (35, 36). Moreover, recombinant TNF α has been shown to inhibit the proliferation of EBV-transformed B cells (37). It is therefore possible that EBV acquired the ability to shut down TNF α synthesis and thus avoid this cytokine's inhibitory effect, as we have already suggested (21). On the other hand, in HSV-1-in-

fectured PBMC cultures, TNF α production started at day 5 postinfection and increased significantly by day 7. As observed in the case of HSV-1-induced IL-6 production, no TNF α synthesis was detected in cell culture supernatants within 3 d after HSV-1 infection. A previous report showed that exogenous TNF α by itself has no inhibitory effects on HSV-1 replication (38). The presence of TNF α protein detected after 5 d of culture indicates that HSV-1 may be insensitive to the antiviral effect of TNF α .

The experiments performed with fractionated cell populations indicate that both viruses directly interact with monocytes/macrophages to induce IL-6 synthesis, and no other cell populations are required for such induction. In fact, larger amounts of IL-6 protein were detected in culture supernatants of enriched monocytic cell populations. This is in contrast with TNF α , where comparable levels of this protein were measured in enriched B and monocytic cell populations after infection with HSV-1. The observation that no TNF α protein was detected in EBV-infected cell cultures suggests that EBV is a strong inhibitor of this cytokine.

Our results showing that UV-irradiated (and not heat-inactivated) EBV and HSV-1 preparations can induce IL-6 and TNF α , respectively, support the hypothesis that a viral struc-

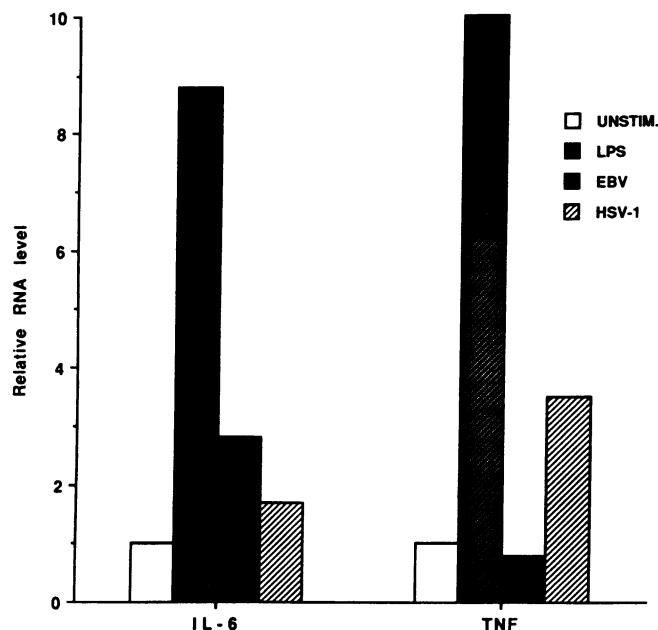


Figure 6. Relative RNA level of EBV- and HSV-1-induced cytokines. Results are expressed in fold increase of RNA levels quantitated relative to the GAPDH levels in each sample and compared to the RNA transcripts of unstimulated cells. Quantification was determined by densitometric scanning of exposed gels.

tural protein(s) or conformationally intact virions are responsible for the induction of these cytokines in the present virus-PBMC system.

The mechanisms of cytokine gene activation/inhibition by viruses are still unknown. By using the sensitive PCR amplification technique, we have demonstrated here that EBV and HSV-1 act differently to transcriptionally regulate IL-6 and TNF α expression in infected cells. In fact, EBV, as a strong inducer of IL-6, also induced larger amounts of IL-6 mRNA transcripts as compared to HSV-1. In contrast, much higher levels of TNF α mRNA were detected in HSV-1-infected cells than in EBV-infected cells. These observations suggest a relationship between cytokine mRNA levels and virus-induced protein (i.e., IL-6 and TNF α) secretion by PBMC. The molecular mechanisms used by EBV and HSV-1 to regulate the synthesis of IL-6 and TNF α are still unknown. It was previously shown that EBV and HSV-1 can respectively bind to and replicate in monocytes/macrophages (21, 39). To date, our attempts to demonstrate EBV replication in monocytic cells have produced negative results. Interestingly, these cells are known to express adhesion molecules on their surface membrane and a recent report has documented the ability of these adhesion molecules to transmit the necessary signals for release of monokines (40). It may therefore be possible that these herpesviruses, while binding to the respective cell surface receptors, interact with the adjacent adhesion molecules to trigger monokine synthesis. It is thus likely that the binding of the virus to its receptor on the cell surface leads to the activation of a transductional pathway involved in the modulation of the cytokine gene expression in the present viral system. In support of this assumption is the above finding that UV-inactivated EBV and HSV-1 preparations are effective in inducing IL-6

and TNF α , respectively, and that virus infectivity is not essential for this process. The fact that the counterpart heat-inactivated viral preparations had no effect on the synthesis of these cytokines would suggest that the heat treatment had a denaturing effect on viral attachment molecules likely rendering them ineffective as ligands to transduce the required signal(s). In any event, further research is needed to delineate the precise molecular events in this process.

The present results clearly illustrate the differential effects of EBV and HSV-1 infections on the immune system, specifically in regards to production of monocyte-derived cytokines. The stronger IL-6 production detected in EBV-infected PBMC cultures infer that this cytokine may be used as a growth factor by EBV-infected cells. On the other hand, HSV-1 could be insensitive to the effect of TNF α as judged by the presence of this cytokine in HSV-1-infected cultures. This is in contrast to the effect of TNF α on EBV (37).

Lastly, although the present data demonstrate that human herpesviruses can modulate cytokine gene expression or synthesis, our results may also be pertinent to understanding the eventual contribution of herpesviruses to both the activation of replication and pathogenesis of other viruses. For example, both IL-6 and TNF α are known to activate the replication of the human immunodeficiency virus (41). Because herpesviral infections can be rampant in patients in various states of human immunodeficiency virus-associated immunodeficiency, it is conceivable that herpesviruses may further contribute to the immunopathological process through cytokine induction.

In conclusion, although both EBV and HSV-1 belong to the same virus family, they seem to have evolved differently in the way they interact with the immune system for inducing or inhibiting certain cytokines. The latter, likely destined initially for cell-to-cell communication, might have become molecules utilized by viruses to disrupt immune responses and favor virus survival. However, more studies are necessary to better understand the various mechanisms by which viruses modulate the immunoregulatory pathways.

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