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Induction of Interleukin-6 after Stimulation of Human B-Cell CD21 by Epstein-Barr Virus Glycoproteins gp350 and gp220

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The cellular receptor for Epstein-Barr virus (EBV) is the type 2 complement receptor, CD21. At initial infection, EBV virion glycoproteins gp350 and gp220 bind to CD21. We report here that the cross-linking of CD21 by gp350/220 results in increased amounts of interleukin 6 (IL-6) RNA and IL-6 protein. This effect could be blocked with anti-gp350/220 and anti-CD21 monoclonal antibodies. Induction of IL-6 in B cells by EBV could be mimicked by treatment with the protein kinase C (PKC) activator phorbol 12,13-dibutyrate but not with the calcium ionophore ionomycin. IL-6 induction by EBV was inhibited with the PKC-specific inhibitor bisindolylmaleimide or the protein tyrosine kinase inhibitors methyl 2,5-dihydroxycinnamate and herbimycin A, indicating that the induction of IL-6 following CD21 cross-linking is mediated through PKC- and protein tyrosine kinase-dependent pathways.

Epstein-Barr virus (EBV) is the causative agent of infectious mononucleosis and is etiologically associated with African Burkitt's lymphoma, as well as nasopharyngeal carcinoma, Hodgkin's lymphoma, and non-Hodgkin's B-cell lymphomas found in immunosuppressed individuals (18). EBV exhibits a unique tropism for cells which express the complement C3d receptor, CD21 (33). In vitro infection of CD21-expressing human B lymphocytes with EBV results in a state of continuous B-cell growth termed immortalization (23). At initial infection, the major EBV outer envelope glycoproteins 350 and 220 (gp350/220) adsorb to CD21 and form a cap prior to endocytosis (33). Cap formation is the characteristic consequence of cross-linking surface proteins and is generally associated with activation of the cell following its induction (6). Interestingly, EBV mediates cocapping of CD21 and surface immunoglobulins (sIg) (33), a finding which suggests that EBV may sequester and activate pathways utilized during normal B-cell receptor activation. Additionally, CD21 is part of the cell surface complex (CD19–CD21–CD81–Leu-13) found to modify sIg-mediated signals (22). Binding of EBV to CD21 results in increased blast formation, cell adhesion, surface CD23 expression, and increased RNA synthesis (10, 33). Previous studies have also shown changes in both intracellular Ca^{2+} levels and phosphorylation of the tyrosine kinase *lck* following CD21 cross-linking (3, 5). From these studies, it was postulated that the quiescent B cell, said to be in the Go_q phase, is induced by gp350/220 cross-linking of the CD21 molecule to progress to an activated or Go_a state. This, however, does not yet commit the B lymphocyte to enter the cell cycle. Following virus internalization and uncoating, EBV-infected B cells sequentially express a limited number of EBV-encoded gene products associated with the latent viral cycle (2).

Recently, interleukin 6 (IL-6) has been demonstrated to be a paracrine or autocrine growth factor for EBV-immortalized B cells, resulting in increased immunoglobulin (Ig) production and B-cell immortalization (1, 41). In vitro EBV-immortalized B cells secrete IL-6 into the culture supernatant, express the IL-6 receptor, and can use IL-6 as an autocrine or paracrine growth factor (36). If IL-6 is expressed at high levels in EBV-immortalized cells, it will promote tumor formation by impairing the activity of natural killer (NK) cells (32). Given the capability of IL-6 to promote B-cell growth and the association of IL-6 with potential immune suppression during B-cell immortalization and B-cell lymphomagenesis, studies aimed at understanding how EBV and its gene products induce IL-6 expression are both important and timely.

In order to verify whether a significant number of EBV-immortalized B cells express IL-6 in vitro compared with uninfected B cells, we measured the levels of extracellular IL-6 bioactivity from B-cell clones derived by immortalization with the EBV strain B95-8 and cultured for less than 3 months (n = 25), B cells derived from peripheral blood of patients with lymphoproliferative disease (n = 4), and cord blood B cells immortalized with orally derived EBV (n = 3). Several EBV-negative Burkitt's lymphoma B-cell lines were used as negative controls (n = 4). IL-6 biological activity from B-cell cultures seeded at 0.5×10^6 cells per ml and cultured for 3 days was measured by using an IL-6 growth-dependent cell line, 7TD1 (CRL 1851; American Type Culture Collection, Rockville, Md.) (26). Briefly, 7TD1 cells (2×10^3 cells per 96-well plate), seeded in Iscove's modified Dulbecco's medium (Sigma, St. Louis, Mo.) supplemented with 10% fetal bovine serum (P.A. Biologicals, Sidney, Australia) and 5×10^{-5} M β-mercaptoethanol, were cultured with serial dilutions of IL-6 containing supernatant for 72 h at 37°C. During the final 4 h of culture, 7TD1 cells were pulsed with [^{3}H]thymidine (2.5 μCi/ml, 6.7 Ci/mmol; DuPont Canada, Mississauga, Ontario, Canada) and harvested with a TomTec cell harvester (Hamden, Conn.) and assayed for [^{3}H]thymidine incorporation with a 1405 microbeta plate liquid scintillation counter (Wallac, Turku, Finland). One unit of bioactivity in this assay is defined as the activity inducing half-maximal proliferation of 7TD1 cells. Recombinant IL-6 (R&D Systems, Minneapolis, Minn.) was used as a posi-

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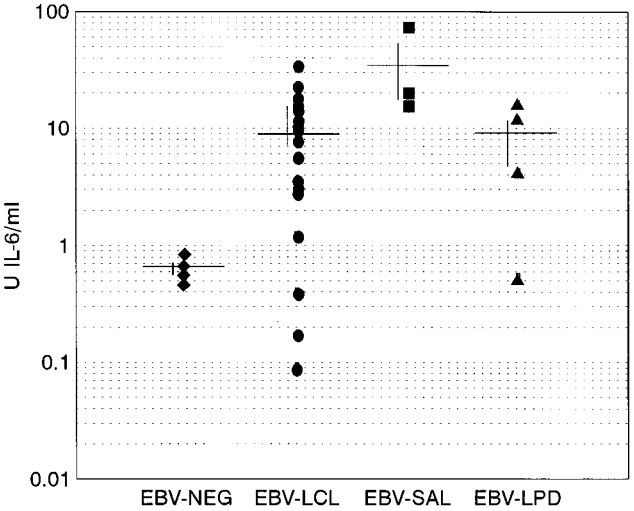

FIG. 1. Expression of IL-6 in EBV-immortalized B cells. B cells recently immortalized with EBV B95-8 (●), cord blood B cells immortalized with salivary EBV (EBV-SAL) (■), and B-lymphoblastoid cell lines (LCL) derived from peripheral-blood B cells obtained from patients with lymphoproliferative disease (LPD) (▲) were assayed for the expression of IL-6. EBV-negative (EBV-NEG) Burkitt's lymphoma cell lines BJA-B, BL41, BL40, and Louckes, which express little or no IL-6, were used as the baseline for non-EBV-induced IL-6 7TD1 proliferation (◆). Average values for IL-6 expression with the corresponding SEM are indicated by the horizontal and vertical bars, respectively.

tive standard. As shown in Fig. 1, over 90% (29 of 32) of the EBV-immortalized B cells expressed IL-6 bioactivity at levels which were at least twofold higher than those seen in the four EBV-negative Burkitt's lymphoma cell lines. The Burkitt's lymphoma cell lines displayed bioactivities of 0.67 ± 0.07 U of IL-6 per ml (mean ± standard error of the mean [SEM]). The average level of IL-6 expression for the 25 EBV-immortalized LCLs was 9.0 ± 1.64 U/ml. The levels of IL-6 expression for EBV cell lines derived by using the oropharyngeal virus and spontaneous lymphoblastoid cell lines derived from lymphoproliferative disease transplant patients were 35.9 ± 18.0 U/ml and 8.41 ± 3.64 U/ml, respectively. When we ranked the relative IL-6 expression in the EBV-positive and EBV-negative populations by Mann-Whitney statistical analysis, we found them to be statistically different populations ($z = 2.77$, exceeding the minimal value, $z_{0.005} = 2.58$) (45).

In order to determine the onset of IL-6 expression during EBV immortalization, highly purified B cells obtained following Ficoll-Hypaque density gradient centrifugation and CD19-Dynabead affinity purification (Dynal, Success Lake, N.Y.) were infected with EBV (strain B95-8) and sequentially assayed over a 3-day period for IL-6 RNA transcription by reverse transcriptase PCR (RT-PCR) (17) or for IL-6 protein expression by biological assay. For RT-PCR, B cells were harvested at hourly intervals, washed with phosphate-buffered saline (PBS) and lysed with Tri-Reagent (MRC, Cincinnati, Ohio). Total RNA was precipitated at −20°C with equal volumes of isopropanol, pelleted by centrifugation (14,000 × *g*), washed with 70% ethanol, and resuspended in water. Equal volumes of RNA were reverse transcribed with random primer (Gibco-BRL, Burlington, Ontario, Canada) and Moloney murine leukemia virus RT (Gibco-BRL) and then subjected to a 30-cycle PCR amplification for IL-6 or a 25-cycle PCR amplification for β-actin. In order to measure IL-6 mRNA, as well as actin, in a semiquantitative manner, we performed serial dilutions of the β-actin mRNA from specimens with the most abundant PCR signal (determined by prior RT-PCR runs). We

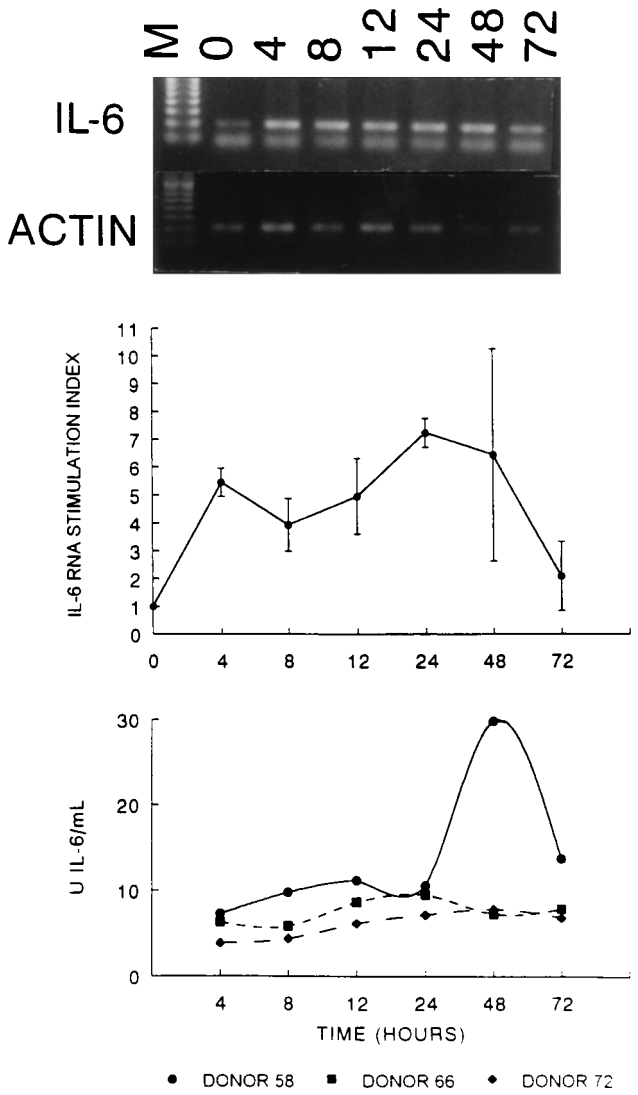

FIG. 2. Time course of B-cell IL-6 RNA and protein expression following EBV infection. Affinity-purified B cells from three separate donors were infected with EBV. IL-6 RNA and protein levels were sequentially assayed over a 72-h period by RT-PCR and by IL-6 biological assay, respectively. (Top) A representative RT-PCR gel is shown. PCR fragment size was determined by using a 100-bp DNA molecular size ladder (M). (Middle) The average IL-6 RNA induction levels ± SEM were plotted for the three separate B-cell donor populations. IL-6 RNA induction was normalized against β-actin. Time zero IL-6 and β-actin RT-PCR gel lane intensities were given an arbitrary value of 1. (Bottom) IL-6 protein expression was also plotted by using IL-6 derived from the same B-cell culture supernatants.

then obtained a relative actin abundance value from the dilution curve for the particular experimental RNA set and the induced IL-6 RNA level for each experiment (25). Primer sequences used for IL-6 and actin were based on previously published sequences (35). Although there was some variation as to the EBV responsiveness for each of the three donors, as shown pictorially for a representative experiment (Fig. 2, top) and graphically as the average IL-6 RNA induction levels (± SEM) from three separate experiments (Fig. 2, middle), EBV was shown to induce detectable IL-6 RNA signals at the earliest times of measurement (4 h postinfection), and these were henceforth maintained at elevated levels. During the 72-h period, EBV induced IL-6 levels to a sevenfold maximum (Fig. 2,

middle). Comparable temporal findings, as measured in the culture supernatants, were also obtained at the protein level for IL-6 (Fig. 2, bottom). IL-6 activity was detected in the culture supernatants as early as 4 to 12 h postinfection and continuously increased throughout the entire 72 h of culture, such that the IL-6 level increased from 5.8 ± 1 U/ml at 4 h to a maximum of 12.3 ± 6.9 U/ml at 24 to 48 h.

Since IL-6 has been shown to be important in EBV immortalization (41) and IL-6 RNA is expressed quite early in viral infection, we attempted to determine whether the simple act of EBV binding was sufficient to induce IL-6 expression. In order to eliminate any potential contribution by a viral gene in the induction of IL-6, we used two methods of EBV inactivation, i.e., UV treatment and psoralen treatment. UV treatment is the standard method for blocking viral replication and transcription (9), but to further control for any possible leakage of EBV transcription following UV treatment, psoralen was also used. Psoralen covalently cross-links GC pairs in the DNA double helix but does not bind protein. Thus, psoralen irreversibly inhibits EBV gene transcription without grossly affecting virion protein structures (7). Concentrated EBV B95-8 virus stocks, which were prepared from 14-day-old B95-8 cell culture supernatants, were inactivated by a 30-min UV irradiation procedure using a laminar flow hood germicidal lamp placed 60 cm above the EBV sample. EBV was inactivated by psoralen cross-linking by initially mixing a virus stock concentrated 50-fold with 90 μg of psoralen (Sigma) per ml and then subjecting the mixture to UV cross-linking using a long-wave UV lamp placed 3 cm above the virus sample for 30 min (11). Psoralen concentrations used were estimated to be 2,500-fold in excess of EBV DNA concentrations (assuming 170 kbp per EBV particle and 10^6 particles per 1 ml of virus stock solution). During the inactivation processes, the virus samples were maintained on ice. Thirty minutes of UV or psoralen treatment was sufficient to inhibit greater than 88 and 90%, respectively, of EBV's B-cell mitogenic activity (31a).

Purified peripheral blood B cells were cultured for 3 days with 1/10 of a volume of concentrated EBV, 1/10 of a volume of UV-treated EBV, or 1/10 of a volume of psoralen-treated EBV in Iscove's modified Dulbecco's medium complete media. B cells were also incubated with Sepharose-coupled anti-IgM (Bio-Rad, Mississauga, Ontario, Canada), which served as a B-cell mitogen. As shown in Fig. 3A, untreated EBV, UV-irradiated EBV, and psoralen-treated EBV were all capable of binding to the B cell and inducing significant and comparable levels of IL-6 in the culture supernatant (31 U/ml). This was in contrast to the case with untreated B cells, which expressed only 7 U of IL-6 per ml. The IL-6 levels induced by the various EBV forms were similar to those seen following B-cell sIgM cross-linking (43 U/ml). The ability of the virus to induce IL-6 appeared specific for EBV. When concentrated UV-inactivated virus was first incubated with purified anti-gp350/220 neutralizing monoclonal antibody, 72A1 (HB 167; American Type Culture Collection) (12), or with anti-CD21 monoclonal antibodies, HB-5 (37) and OKB-7 (Becton Dickinson, Mountain View, Calif.), for 1 h at 4°C prior to addition to the purified B cells, IL-6 induction could be blocked. IL-6 levels were reduced by 70% with the addition of the gp350/220 neutralizing monoclonal antibody 72A1, which blocks binding of gp350/220 to CD21 (34). Also, IL-6 levels were reduced by 75% with the anti-CD21 monoclonal antibody OKB7, which blocks EBV-CD21 binding (21), and IL-6 levels were reduced by 55% with the anti-CD21 monoclonal antibody HB-5, which affects CD21 B-cell signaling (43) (Fig. 3B). On the other hand, EBV's induction of IL-6 was not blocked with an irrelevant monoclonal antibody, OKT3 (Becton Dickinson), which recognizes the T-cell antigen CD3.

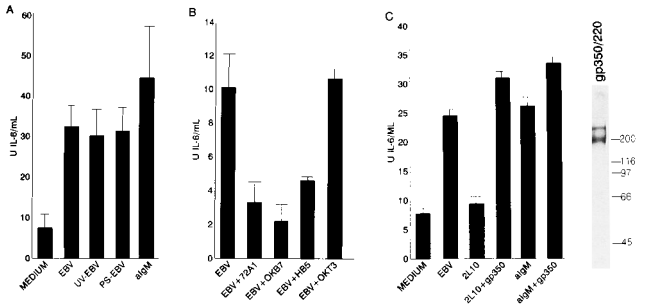

FIG. 3. Induction of IL-6 by EBV and EBV gp350/220. (A) Purified peripheral blood B cells (2.5×10^6/ml) were cultured for 3 days with either medium alone, 1/10 of a volume of concentrated EBV (EBV), 1/10 of a volume of UV-treated EBV (UV-EBV), 1/10 of a volume of psoralen-treated EBV (PS-EBV), or 1/20 of a volume of Sepharose-coupled rabbit anti-human IgM (aIgM). (B) Purified peripheral blood B cells (6.5×10^5/ml) were cultured for 3 days with concentrated UV-treated EBV (EBV) or UV-treated virus which had previously been incubated at 4°C for 1 h prior to B-cell addition with 5 μg of anti-gp350/220 neutralizing monoclonal antibody (72A1), anti-CD21 monoclonal antibody (OKB-7 or HB-5), or anti-CD3 monoclonal antibody (OKT3). Following several washes with PBS, cells and virus were cultured for 72 h. (C) Purified peripheral blood B cells (2.5×10^6/ml) were cultured for 3 days with UV-treated EBV (EBV), Sepharose-coupled rabbit anti-mouse Ig–anti-gp350/220 monoclonal antibody (2L10), Sepharose-coupled rabbit anti-mouse Ig–2L10 and purified gp350/220 (2L10+gp350), Sepharose-coupled rabbit anti-human IgM (aIgM), or Sepharose-coupled rabbit anti-human IgM and the gp350/220 Sepharose complex (aIgM+gp350). The purity of gp350/220 used in culture was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and silver staining (shown on the right). Results represent the average IL-6 concentrations ± SEM for three separate experiments.

In order to determine whether the major virion glycoproteins, gp350/220, which have previously been shown to bind directly to the CD21 receptor (33), were capable of inducing IL-6 in B cells alone, and not in conjunction with another virion surface protein, B cells were incubated with purified gp350/220 coupled to Sepharose. gp350/220-coupled Sepharose was generated by initially incubating the gp350/220 monoclonal antibody 2L10 with rabbit anti-mouse Ig-coupled Sepharose (Bio-Rad) and then incubating it with purified gp350/220 (34). All incubation steps were carried out at 4°C for 1 h with several interim PBS washes to remove unbound ligands. Purified gp350/220 was obtained by affinity chromatography and gel filtration from GH3Δ19 culture supernatant as described previously (34). The gp350/220 preparation was checked for purity by silver staining (24). The 2L10 monoclonal antibody, which recognizes gp350/220, has previously been shown not to block EBV gp350/220 from binding to CD21 (34). As seen in Fig. 3C, purified gp350/220 was capable of inducing IL-6 in the B-cell culture to levels comparable to that of virus, whereas Sepharose coupled with 2L10 monoclonal antibody alone was not. When coincubated with anti-IgM-coupled Sepharose, gp350/220 appeared to slightly enhance IL-6 production (7%) over the level seen for anti-IgM alone, suggesting that the gp350/220-CD21 complex may complement signals similar to that involved with sIg or may serve to modify sIg B-cell signaling.

Since IL-6 is induced following CD21-gp350/220 interaction and since the signaling mechanism(s) for IL-6 induction by CD21 is presently unknown, experiments were performed to determine a possible CD21 signaling pathway for IL-6 induction. Two signals, namely Ca^{2+} and protein phosphorylation, are known to be important in B-cell physiology and proliferation (8). Experiments were performed to determine whether

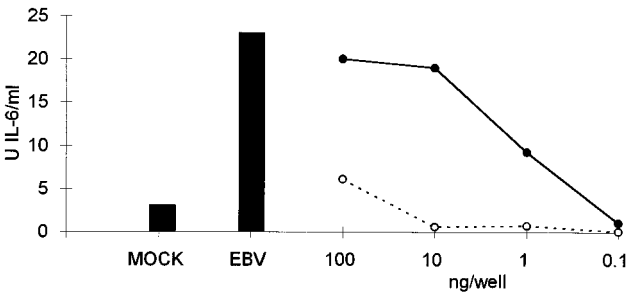

FIG. 4. IL-6 induction with ionomycin or the phorbol ester in B cells. Affinity-purified B cells (10^4) were mock infected, infected with 1/10 of a volume of psoralen-cross-linked EBV, or treated with various amounts of phorbol 12,13-dibutyrate (●) or ionomycin (○). After 72 h of incubation, B-cell culture supernatants were analyzed for IL-6 bioactivity. Proliferative responses by 7TD1 cells due to endogenous phorbol 12,13-dibutyrate or ionomycin were subtracted from the B-cell IL-6-induced proliferative responses.

Ca^{2+} or protein phosphorylation could mimic the effects of virus binding. As indicated in Fig. 4, the phorbol ester phorbol 12,13-dibutyrate (Gibco-BRL), which stimulates protein kinase C (PKC), was capable of inducing significant increases in IL-6 expression in purified B cells, whereas no significant IL-6 induction was seen when comparable amounts of ionomycin (Sigma), which increases intracellular Ca^{2+} levels, were used (Fig. 4). In order to confirm that PKC and/or other protein kinases may be involved in IL-6 induction following CD21 signaling, a series of protein kinase inhibitors were added to purified B cells concurrently with the addition of psoralen-cross-linked EBV. If a particular kinase is involved in the induction of IL-6 following CD21 cross-linking, then the addition of the corresponding kinase inhibitor may reduce or abrogate IL-6 expression. As shown in Table 1, addition of the PKC-specific inhibitor bisindolylmaleimide (Boehringer Mannheim, Laval, Québec, Canada) inhibited IL-6 induction following EBV infection by 95.5%. Two other protein tyrosine kinase (PTK) inhibitors, methyl 2,5-dihydroxycinnamate and herbimycin A (Gibco-BRL), also significantly inhibited IL-6 expression (93.3 and 98.8% inhibition, respectively). The other PTK inhibitors tested either showed a modest inhibition of EBV-induced IL-6 expression (39.8 to 58.1% inhibition) or demonstrated no inhibition at all or a slight augmentation of IL-6 expression in EBV-infected B cells (Table 1). The inhibition of IL-6 expression by bisindolylmaleimide, methyl 2,5-dihydroxycinnamate, and herbimycin A was not due to kinase inhibitor cell toxicity, since the B cells which showed a significant reduction in IL-6 levels had cell viability counts comparable to those observed with the other kinase inhibitors (averages of 72.7 and 79.1%, respectively) (Table 1). Thus, IL-6 expression following gp350/220 binding may require protein kinase phosphorylation, probably acting through pathways which involve PKC and certain PTKs.

Two of the hallmarks of in vitro EBV B-cell infection are continued B-cell growth and increased Ig synthesis. These characteristics are believed to result from the expression of a limited number of virally encoded genes which mimic signals utilized in normal B-cell activation. By substituting or supplying the B-cell growth components necessary for a quiescent B cell to progress beyond the Go activated state, EBV infection may render B cells capable of continuous cell growth and maturation (39). Our results indicate that the cross-linking of the CD21 receptor with gp350/220 results in a significant up-regulation of IL-6. This effect could be blocked with specific gp350/220 or CD21 monoclonal antibodies. Furthermore, we could mimic the induction of IL-6 by the addition of the phorbol ester phorbol 12,13-dibutyrate but not with the calcium ionophore ionomycin. Finally, we could inhibit IL-6 expression following EBV infection plus treatment with the PKC-specific inhibitor bisindolylmaleimide and several PTK inhibitors, suggesting that B-cell induction of IL-6 following CD21 cross-linking is mediated through PKC- and PTK-dependent pathways and not through signaling pathways which are induced by Ca^{2+}.

We have previously demonstrated that gp350/220, as well as other ligands to the CD21 molecule, resulted in the colocal-

TABLE 1. Inhibition of IL-6 during EBV infection by PKC and PTK inhibitors

Inhibitor (concn)[a]	Site of action (reference)[b]	IL-6 concn (pg/ml)[c]	% EBV induction	% Cell viability (avg ± SEM)[d]
Mock infection		0.28 ± 0.27		75.7 ± 9.8
None		28.69 ± 1.3		79.3 ± 3.7
Bisindolylmaleimide (5 μM)	Inhibitor of PKC isoforms α, β1, 11, γ (42)	1.30 ± 0.08	4.5	76 ± 8.1
2-Hydroxy-5-(2,5-dihydroxybenzyl) aminobenzoic acid (50 nM)	Inhibitor of EGF receptor-associated PTK (44)	12.02 ± 0.94	41.9	84.7 ± 6.4
Methyl 2,5-dihydroxycinnamate (25 μM)	Inhibitor of EGF receptor-associated PTK (29)	1.91 ± 0.20	6.7	71 ± 7.5
Genistein (92 μM)	Inhibitor of ATP binding to tyrosine-specific protein kinase (27)	17.27 ± 0.67	60.2	75.3 ± 9.1
Herbimycin A (8.7 μM)	Inhibitor of PTKs (13)	0.35 ± 0.04	1.2	71 ± 7.2
Lavendustin A (65 μM)	Competitive inhibitor of ATP binding to EGF receptor-associated PTKs (16)	16.71 ± 0.96	58.2	71 ± 8.8
Reduced carbamoyl methylated-lysozyme (20 μM)	Inhibitor of insulin-associated kinase (14)	61.55 ± 5.0	214	72.7 ± 5.9
Staurosporine (50 nM)	Inhibitor of phospholipid calcium ion-dependent protein kinase (15)	46.92 ± 3.3	163	83.3 ± 1.6
Tyrphostin (100 μM)	Inhibitor of substrate binding to EGF-associated PTK (19)	33.61 ± 2.20	117	87.3 ± 4.2

[a] CD19 affinity-purified B cells from three separate donors which were seeded at 10^5 cells per ml in Iscove's modified Dulbecco's medium–10% fetal calf serum and 1/10 of a volume of concentrated psoralen-cross-linked B95-8 virus stock were cultured in the presence or absence of the kinase inhibitors.
[b] EGF, epidermal growth factor.
[c] IL-6 culture supernatant concentrations were determined by enzyme-linked immunosorbent assay (R&D Systems). Data are averages ± SEM.
[d] Cell viability was determined by trypan blue exclusion.

ization of sIg, leading to B-cell activation (33). CD21, in association with CD19, CD81, and Leu-13, forms a complex which is postulated to be an important modulator of sIg signaling and B-cell immunoresponsiveness (38). Work by Carter and Fearon (3) indicates that this receptor complex greatly enhanced B-cell activation, such that coligation of the CD19 and sIg reduced by twofold the number of B-cell Ig receptors that must be ligated in order to induce B-cell proliferation (3). Further, administration of a soluble CD21-IgG1 fusion protein suppressed in vivo immune responses to thymus-dependent antigens. This presumably occurred by the blocked association of sIg and the CD21-CD19 receptor complex (4). The sIg activation of B cells through the CD21–CD19–CD81–Leu-13 complex is thought to be mediated mainly through the cytoplasmic tails of CD19 and CD81 (38). Cross-linking of the CD19 receptor results in increased PTK activity, leading to the phosphorylation of CD19 and other cytoplasmic and cell surface proteins and ultimately to activation of phospholipase C, inositol phospholipid turnover, Ca^{2+} mobilization, stimulation of serine-specific protein kinases (including PKC), and activation of NF-κB and c-*fos* (20, 38). Cross-linking of CD81, in conjunction with Leu-13, also results in increased phosphorylation of cellular proteins (31). From these results, one may hypothesize that the CD21-CD19-CD81-Leu-13 complex synergizes with sIg signals and amplifies the B-cell immune response. Since IL-6 is an important cytokine in B-cell maturation and abrogation of CD21 responses results in impaired B-cell immune function (4), our results suggest that one important way in which CD21 acts to enhance B-cell growth and sIg receptor function is through the induction of IL-6.

EBV has been implicated as a cofactor in the establishment of a variety of B-cell lymphomas (18). The exact mechanism(s) for the development of these lymphomas is, as of yet, unclear. In healthy individuals infected with EBV, elimination of EBV-positive B cells is initiated by NK cells followed by EBV-specific cytotoxic T cells (28). Impairment of cytotoxic T cell or NK cell function in an immunosuppressed patient could provide an opportunity for EBV lymphomas to develop. Our previous results indicated that IL-6 can promote the expansion of the EBV-infected B-cell pool both by supplying autocrine growth factors and by impairing immune cell cytolytic function (32, 36). Support for an analogous mechanism for lymphoma development in immunosuppressed patients is suggested by studies which have demonstrated that patients with elevated levels of IL-6 in serum show an increase in the number of EBV-infected peripheral blood B cells and a corresponding increased risk for developing lymphoproliferative disease (30, 40). Because the mechanisms of cytokine regulation during B-cell antigen activation and EBV B-cell lymphomagenesis are still undefined, our observations demonstrating the induction of IL-6 by gp350/220 will, we hope, further the understanding of B-cell physiology and of the EBV B-cell lymphomagenesis process. New research efforts can then be directed at strategies to block synthesis of dysregulated cytokines in EBV lymphomas, as well as other cytokine-related cancers.

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