POSTTRANSPLANT patients undergoing prolonged immunosuppressive therapy using agents such as cyclosporine A (CsA) have been reported to have increased incidence of Epstein-Barr virus (EBV)-associated lymphoproliferative disorders.1 For patients undergoing immunosuppressive therapy, EBV B-cell non-Hodgkin’s lymphomas (NHL) represent the second most common malignancy, constituting 26% of all cancers occurring in transplant recipients.2 These EBV B-cell lymphomas are classified as NHL of high grade type and exhibit morphologic changes ranging from polymorphic diffuse B-cell hyperplasia to diffuse polymorphic B-cell lymphoma.3 These lymphomas characteristically display rapid onset, aggressive behavior, predilection for extranodal sites of presentation, and partial or complete regression after reduction or withdrawal of the immunosuppressive therapy.

The increased frequency of lymphoproliferative disorders after CsA treatment and the almost invariable demonstration of EBV DNA or EBV-encoded gene products have suggested important clues to an understanding of the pathology of NHL in transplant patients. After use of CsA for prophylaxis and treatment, CsA-associated NHL represent 8% to 30% of all de novo malignancies, as compared with 0% to 12% for patients undergoing other conventional immunosuppressive regimes.3,4 Comparative studies of CsA versus non-CsA forms of immunosuppression indicate that CsA-related tumors develop more rapidly, on average within 12 months of grafting, compared with tumors occurring in non-CsA-treated patients; the latter may exhibit tumor onsets of more than 16 years after transplant surgery.1 In those B-cell lymphomas that were examined EBV was detected in a majority of the cases.5

Although the mechanism of action of CsA is not well understood, CsA has been shown to act on a number of different cell types, thus producing multiple effects. CsA’s utility in organ transplantation stems principally from its ability to inhibit early step(s) in T-cell activation, leading to repression of early activation genes such as interleukin-2 (IL-2).6

Patients undergoing immunosuppressive therapy have been shown to have elevated levels of IL-6 in the serum.7 One study found a strong correlation in the incidence of posttransplant EBV-positive lymphomas and markedly increased levels of serum IL-6.8 IL-6 has recently been shown to act as an autocrine or paracrine growth factor and stimulator of immunoglobulin production in EBV-immortalized B cells.8,9 If produced at high levels, IL-6 is capable of promoting tumor formation in immunocompromised hosts, by acting principally to inhibit the host’s natural killer cell activity.10

The principal aim of this study was to analyze the contribution of CsA in the outcome of EBV B-cell interactions by examining CsA’s capacity to (1) promote B-cell immortalization, (2) promote the viral lytic cycle, and (3) stimulate the EBV B-cell growth factor, IL-6, in peripheral blood mononuclear cells (PBMC).

MATERIALS AND METHODS

EBV-infection and CsA treatment of PBMC. PBMC were obtained from healthy donors after separation on Ficoll-Hypaque density gradients. (Pharmacia [Canada] Inc, Baie d’Urfe, Quebec), fractionation by plastic adherence11 (monocytes, 85% ± 10.4% SEM: CD11b+, anti-OKM1, Becton Dickinson, San Jose, CA; and 8% ± 1.0% T- and B-cell contaminant), and rosetting with 2-aminohexyl-sulfochromium bromide hydrobromide (AET)-treated sheep red

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blood cells (SRBC; T cells, 87% ± 6.4%: CD3+, anti-Leu 4, Becton Dickinson; and 21% ± 8.6% B-cell and monocyte contaminants), or as nonrosetting cells (B cells, 60% ± 14%; CD19+, anti-Leu 12, Becton Dickinson; 46% ± 8.5% non-B cell, monocyte, T-cell contaminant). In experiments where IL-6 expression was analyzed from purified B-cell populations, B cells were isolated using CD19-dynabeads (Dynal, Success Lake, NY) as recommended by the manufacturer. Cells were found to be 95% ± 2.4% CD19+, 4.3% ± 2.6% CD3+, and 0.94% ± 0.032 CD14+ (Leu M3, Becton Dickinson). The cell purity is expressed as the average ± SEM based on FACS analysis from three separate experiments. Cells to be tested for IL-6 expression were seeded at 2 to 5 × 10⁶ cells per milliliter RPMI 1640 (GIBCO-BRL, Bethesda, MD) supplemented with 10% heat-inactivated fetal calf serum (ICN, Costa Mesa, CA), 20 mM HEPES (GIBCO-BRL, Gaithersburg, MD), 0.1 g/L non-essential amino acids (GIBCO-BRL, Gaithersburg, MD) and 100 units/ml penicillin, 100 ng/ml streptomycin (GIBCO-BRL, Gaithersburg, MD). Cells were washed several times with phosphate-buffered saline (PBS), lysed in gel sample buffer (1 × 10⁴/mL), and resolved by SDS-PAGE using a 10% gel. Cell proteins were blotted onto Immobilon (Millipore Corp, Bedford, MA), and EBV-lytic antigens were detected using high-titer VCA-reactive human serum or anti-EBV early antigen-diffuse (EA-D) monoclonal antibody, R3 (a gift from G. Pearson, Georgetown University, Washington, DC), and ECL western blotting detection reagents (Amersham, Buckinghamshire, UK).

Assay for IL-6 bioactivity. Levels of IL-6 released into the culture medium were determined using an IL-6 growth-dependent cell line 7TD1 (CRL 1851; ATCC). The 7TD1 cells (2 × 10⁶ cells per 96-well), which were seeded in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), were cultured for 24 hours. Thirty minutes of UV-treatment was sufficient to inhibit greater than 88% of EBV's B-cell mitogenic activity (J.E.T., unpublished data, April 1994). Monocyte culture supernatant was then assayed for IL-6 using 7TD1 cells.

Transformation of B cells. Peripheral blood B cells, which were purified as described above, were seeded at 100 cells in round-bottom 96-well microplates (Nuncion, Kamstrup, Denmark) and EBV-infected in the presence or absence of varying doses of CsA (250 to 2,000 ng/mL). In other experiments, B cells were seeded at 1,000, 100, or 10 B cells per well in medium containing 600 ng/mL CsA. Cells were cultured for 3 weeks in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum (ICN, Costa Mesa, CA). Wells were visually scored after 3 weeks postinfection for B-cell immortalization by cell outgrowth. Within the range of concentrations of CsA used (250 to 2,000 ng/mL), CsA exhibited an average growth inhibition of 7.6% to 25.5% when tested on three EBV B-cell lines (P3HR-1, B95-8, and EBV-TJ) and one EBV-negative B-cell line (Louckes), as measured by [³H]-thymidine incorporation (J.E.T., unpublished data, June 1994).

Induction of EBV by CsA or IL-6. EBV-immortalized lymphoblastoid cell lines (LCL) derived from peripheral blood B cells and cultured for less than 2 months; long-term EBV-immortalized LCL derived from patients diagnosed with posttransplant lymphoproliferative disease (LPD); long-term cord blood lymphocytes originally immortalized with saliva-derived EBV (gift from C. Alfieri, University of Montreál, Montréal, Quebec); or the EBV-lytic-virus cycle inducible cell lines B95-8 (CRL 1621; ATCC), P3HR-1 (HTB 62; ATCC, Accut, and AG-876) were seeded at a cell density of 1 × 10⁶ cells per milliliter supplemented with either CsA or IL-6 (R&D Systems, Minneapolis, MN) and cultured at 37°C for 3 days. In several experiments P3HR-1 virus lytic-cycle induction with IL-6, anti-human IL-6 neutralizing monoclonal antibody (clone 6708.11; R&D Systems) was also added. Parallel cultures treated with 30 ng/mL O-Tetracainophenol 13-acetate (TPA; Sigma, St. Louis, MO) served as positive controls for lytic virus-producing cells.

Detection of lytic-cycle antigens. Cells were processed for immunofluorescence microscopy after methanol fixation. EBV lytic antigen-positive cells were visualized by initially incubating them with high-titer viral capsid antigen (VCA)-reactive human serum, followed by incubation with fluorescein isothiocyanate (FITC)-conjugated goat-anti-human IgG serum (Cappel Labs, West Chester, PA).

Individual lytic-cycle viral proteins were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and detected by Western blots. Cells were washed several times with phosphate-buffered saline (PBS), lysed in gel sample buffer (1 × 10⁴/mL), and resolved by SDS-PAGE using a 10% gel. Cell proteins were blotted onto Immobilon (Millipore Corp, Bedford, MA, MA), and EBV-lytic antigens were detected using high-titer VCA-reactive human serum or anti-EBV early antigen-diffuse (EA-D) monoclonal antibody, R3 (a gift from G. Pearson, Georgetown University, Washington, DC), and ECL western blotting detection reagents (Amersham, Buckinghamshire, UK).

RESULTS

Effects of CsA on EBV B-cell immortalization. Earlier reports have noted an increase in the number of EBV-immortalized B cells when PBMC were EBV-infected in the presence of CsA. Results obtained using peripheral blood B cells infected with EBV in vitro and cultured for 3 weeks in the presence of CsA showed an increase in the number of EBV-immortalized cells.

B cells that were EBV-infected and seeded at 100 B cells per well in the presence of increasing doses of CsA revealed a 1.7-fold increase in the number of EBV-immortalized B cells, as compared with no CsA treatment (dose range, 500–1,000 ng/mL; Fig 1A). Beyond doses of 1,000 ng/mL, CsA reduced the number of EBV-immortalized cells, possibly due to CsA toxicity. When B cells were seeded with 600 ng/mL CsA at 1,000, 100, and 10 B cells per well, increased EBV-B-cell outgrowths of 0.8–4.3-fold, respectively, were observed, as compared with B cells cultured without CsA (Fig 1B). These results suggest that CsA markedly enhances EBV B-cell immortalization, especially when B cells are present at low cell density (100 to 10 B cells per well).

Recently, IL-6 has been shown to promote EBV immor-
EBV infection induced a 35-fold increase in the amount of IL-6 RNA transcripts after 24 hours of culture. Together, EBV plus CsA at this time demonstrated a 64-fold increase in the level of IL-6 RNA. CsA alone exhibited a 12-fold increase in the level of IL-6 RNA. These data indicate that at 24 hours CsA does not inhibit IL-6 transcription induced by EBV, and that the majority of IL-6 RNA transcription appeared due to the action of EBV.

At 48 hours of culture, EBV plus CsA continued to show elevated levels of IL-6 RNA as compared with untreated cells (eightfold induction), with each demonstrating a 5- and 8.6-fold increase, respectively (Fig 2B). The delayed increase in IL-6 RNA when PBMC were treated with CsA alone would suggest that CsA may be acting either upon an additional cell population different from the EBV-induced population or may act post-infection to enhance expression of IL-6 in the EBV-activated cell population.

By 72 hours of culture, the levels of IL-6 for each of the three treatments, ie, EBV, EBV plus CsA, and CsA alone, demonstrated a 2.9-, 3.4-, and 3.7-fold increase, respectively, as compared with untreated cells, indicating that EBV and CsA, alone and together, continue to stimulate IL-6 expression (Fig 2A).

To determine which cell population was acted on by EBV or CsA, PBMC were separated into monocyte, T-cell, and B-cell populations, cultured for 72 hours with EBV and CsA, and assayed for IL-6 bioactivity. As shown in Fig 3, the induction of IL-6 appears to be caused by different mechanisms in the different PBMC populations. In peripheral blood monocytes, EBV appears to be a strong inducer of IL-6, exhibiting an 8.3-fold increase in IL-6 production. The EBV-induced IL-6 represents the majority of the IL-6 bioactivity, as CsA alone appeared to contribute only 17% of the total monocyte IL-6 bioactivity. T cells appear to be more responsive to CsA for IL-6 production. Of the 3.6-fold total IL-6 bioactivity increase seen with EBV plus CsA, CsA contributed 83% of the total T-cell IL-6 bioactivity. These T cell results are in agreement with those observed in CsA-treated, immortalized cells, and greater than 96% of randomly selected cultured EBV-LCL demonstrated IL-6 bioactivity (30131 units/mL). Although IL-6 has been demonstrated to be an autocrine growth factor for EBV-immortalized cells, and greater than 96% of randomly selected cultured EBV-LCL demonstrated IL-6 bioactivity (30131 units/mL).

**Fig 1.** Effect of CsA on EBV B-cell immortalization and IL-6 expression. (A) B cells that were obtained after Ficoll-Hypaque fractionation, removal of monocytes by plastic adherence, and removal of T cells by AET-SRBC rosetting were seeded at 100 B cells per well in two round-bottom 96-well plates. Relative number of B cells per dilution was determined by FACS. Cells were infected with EBV (strain B95-8) and cultured in RPMI 1640, 10% FCS, with or without CsA. After 3 weeks, wells were visually scored for B-cell immortalization by cell outgrowth. Donor A, (■); donor B, (□). (B) B cells obtained as described above were seeded at 1,000, 100, or 10 B cells per well in two round-bottom 96-well plates, infected with EBV, and cultured with or without 600 ng/mL CsA. After 3 weeks, wells were visually scored for B-cell immortalization by cell outgrowth. Results were plotted as the average ± SEM from three separate experiments. CsA+, (■); CsA−, (□). (C) B cells obtained from three separate donors and recently immortalized (cultured for less than 3 months with EBV strain B95-8 in the presence or absence of CsA) were seeded at 5 × 10⁶ cells per milliliter in RPMI 1640, 10% FBS, and cultured for 4 days. Culture supernatant was assayed for levels of IL-6. Mean ± SEM IL-6 bioactivity levels from CsA-treated (n = 18, ■) and untreated (n = 13, □) were then determined.
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phytohemagglutinin-activated T cells, which also showed an increased expression of IL-6.27 Although B cells, like monocytes, also express IL-6 after EBV infection (1.4-fold) and are not induced to significantly express IL-6 after CsA treatment, they do not appear to be stimulated to the same levels after EBV infection.

Because monocytes have not previously been demonstrated to express significant levels of the EBV receptor, CD21, we wished to determine whether EBV’s induction of IL-6 in monocytes was through a specific virus/monocyte interaction, mediated by the EBV glycoproteins, gp350 and gp220,28 or through an EBV-induced but nonspecific interaction. As shown in Fig 4, exposure of monocytes to either untreated or UV-inactivated EBV resulted in a comparable and significant increase of IL-6 (6.6-fold). The IL-6 induction could be blocked by 46% using the gp350, gp220 specific neutralizing monoclonal, 72A1, but was not blocked by an irrelevant monoclonal, OKT3. OKT3-treated samples exhibited only a 5% difference in IL-6 levels as compared with no antibody treatment.

Induction of the virus lytic-cycle by CsA. Recently, examinations of lymphomas from immunosuppressed individuals have suggested some evidence of viral lytic-cycle gene expression at the tumor site.29,30 To eliminate the effects of
contaminating monocytes and T cells on EBV-infected B cells when measuring the effects of CsA on the EBV-infected B cells, representative EBV-immortalized B-cell lines were used. Experiments were performed to analyze whether CsA had any direct effect on EBV-infected B cells. These included recently immortalized lymphoblastoid cells (less than 2 months in culture), long-term cord blood B lymphocytes originally immortalized with orally derived virus, long-term spontaneous EBV-LCL derived from patients diagnosed with LPD, and four established EBV-producer cell lines: AG-876, Akata, B95-8, and P3HR-1. At the CsA concentration used (500 ng/mL), only the recently EBV-immortalized LCLs were induced to produce viral lytic antigens after CsA treatment (4/11; Table 1). The average induction levels were threefold greater than in untreated cells. The other latently infected cell lines derived from either orally secreted virus or LPD patients revealed no detectable viral lytic-cycle antigens. Analysis of producer cell lines showed induction of viral lytic-cycle antigens by CsA in both B95-8 and P3HR-1 (2.3- and 1.9-fold, respectively). Both these cell lines responded to CsA in a dose-dependent manner, as seen by an increase in the numbers of VCA-positive cells (Fig 5). B95-8 and P3HR-1 cells demonstrated maximum VCA levels of 11.6% to 12.7% and 9.1% to 10.1%, respectively. This represents a 2.8- to 4.7-fold and 3- to 3.5-fold increase in the number of VCA-positive cells, as compared with untreated B95-8 and P3HR-1 cells, respectively. These VCA levels induced in the two cell lines (B95-8 and P3HR-1) are comparable with another well-characterized virus-inducing agent, TPA. TPA-treated cells exhibited a 12.1% to 16% and 6.6% to 12.6% VCA-level increase for B95-8 and P3HR-1, respectively (Fig 5). However, the cell lines AG-876 and Akata failed to be significantly induced by CsA (1.2% over medium, and undetectable, respectively) but were responsive to TPA and anti-IgG, respectively (Table 1).

Analysis of P3HR-1 and B95-8 EBV lytic antigens, such as EA-D, as well as other EBV-lytic antigens in CsA-treated cells showed an increase in expression of both EA-D and other EBV-lytic antigens (Fig 6). CsA induced a 7.5-fold and a 1.5-fold increase of EA-D in both P3HR-1 and B95-8, respectively. Other EBV-lytic protein bands ranging from 25 kD to 32 kD were also induced by 3.5-fold in B95-8 and 2.8-fold in P3HR-1, after CsA treatment (Fig 6). These

### Table 1. Induction of EBV Lytic Infection With CsA and IL-6

<table>
<thead>
<tr>
<th>Medium*</th>
<th>IL-6</th>
<th>CsA</th>
<th>TPA, Anti-IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBMC B95-8 LCL (N = 11)</td>
<td>4.12 ± 0.37</td>
<td>4.03 ± 0.09</td>
<td>4.39 ± 1.7</td>
</tr>
<tr>
<td>LPD LCL (N = 3)</td>
<td>ND</td>
<td>1.04</td>
<td>ND</td>
</tr>
<tr>
<td>Oral EBV LCL (N = 3)</td>
<td>ND</td>
<td>1.03</td>
<td>ND</td>
</tr>
<tr>
<td>P3HR-1</td>
<td>5.9 ± 0.99</td>
<td>16.8 ± 2.6</td>
<td>11.3 ± 2.3</td>
</tr>
<tr>
<td>B95-8</td>
<td>3.4 ± 0.28</td>
<td>5.2 ± 1.0</td>
<td>7.9 ± 1.3</td>
</tr>
<tr>
<td>AG-876</td>
<td>3.3 ± 0.05</td>
<td>7.7 ± 0.9</td>
<td>4.5 ± 0.05</td>
</tr>
<tr>
<td>Akata</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

In the LCL groupings, values are expressed as the number of positive cell lines, percent VCA-positive cells per 200 cells ± SEM. Values for the lytic-inducible cell lines are expressed as the percent VCA-positive cells per 200 cells ± SEM from four different experiments.

Abbreviation: ND, no detectable VCA.

* Cells were incubated with 2 x 10^3 U/mL IL-6, 500 ng/mL CsA, 30 ng/mL TPA, or a 1:50 dilution of sheep anti-human IgG for Akata virus induction (Cappel).
protein bands appeared similar to those present after TPA treatment and were low or absent in untreated cells. The level of lytic proteins were comparable with the increase in VCA-positive cells after CsA treatment (2.1- and 1.5-fold increase in VCA-positive P3HR-1 and B95-8 cells, respectively), suggesting that CsA acted to increase both the number of lytic-antigen positive cells, as well as individual viral-lytic proteins (Fig 6).

**Induction of lytic-cycle antigens by IL-6.** As IL-6 has been shown to be induced after CsA treatment of the various PBMC populations (Fig 2), and earlier studies have demonstrated its role as a B-cell growth and differentiation factor for EBV-immortalized B cells, experiments were performed to determine whether IL-6 was capable of inducing the viral lytic-cycle in EBV-infected B cells. As shown in Table 1, although IL-6 was capable of inducing the viral lytic-cycle in several EBV-latent LCL (6/16), there was no significant level of antigen induction within each of the induced cell lines (<1%). As only those cell lines which were cultured for less than 2 months demonstrated the most significant induction (4/11), this might suggest that long-term culture of EBV-immortalized cells may select for a more tightly-restricted latent phenotype. Analysis of those cell lines which are capable of producing virus showed P3HR-1 to be most affected by IL-6 (2.8-fold induced). This result was also verified by Western blot, where both the expression of EA-D, as well as several other EBV lytic-cycle antigens were increased (6.8-fold for EA-D and 1.8-fold for the 25 kD to 32 kD viral lytic-cycle proteins; Fig 6). To confirm that the induction of the viral lytic antigens was mediated by IL-6, P3HR-1 cells were cultured with IL-6 in the presence or absence of anti-human IL-6 neutralizing monoclonal antibody. As shown in Fig 6, P3HR-1 cultured with either TPA or IL-6 increased both EA-D and VCA levels by 7.5- and 2-fold, or 12- and 3.3-fold, respectively. Addition of neutralizing antibody reduced EA-D and VCA levels in both untreated and IL-6-cultured cells. The reduction of EA-D and VCA by neutralizing antibody in untreated cells was possibly due to the removal of endogenous P3HR-1 IL-6. IL-6 did not increase the level of EA-D or lytic-cycle antigens in B95-8 (Fig 6).

To determine whether TPA’s ability to induce virus may be mediated in part by its ability to act as a B-cell differentiation and/or stimulatory agent, and thus induce endogenous IL-6, several EBV-immortalized LCL exhibiting low (1.2-1.7 U/mL), medium (2.0-2.9 U/mL), or high (3.2-5.4 U/mL) levels of IL-6 were treated with TPA and ionomycin. In addition, P3HR-1, B95-8, or primary B cells were also treated with these agents. TPA and ionomycin were chosen because they act together to mimic normal B-cell activation signals. As shown in Fig 7, TPA induced an average 1.6-fold increase in the level of IL-6 in each of the EBV-immortalized LCL and in P3HR-1, whereas ionomycin treatment showed a slight decrease in IL-6 levels (20% decrease) in these same cell lines. B95-8, which expressed high levels of IL-6 activity without stimulation, was largely unaffected by either TPA or ionomycin. Primary B cells, on the other hand, showed increases in IL-6 levels after treatment with either TPA or ionomycin and their affects appeared additive.

Given the already high levels of endogenous IL-6 produced in B95-8, the lack of virus induction by IL-6 may possibly be due to either a lack of IL-6 responsiveness or an absence of IL-6 receptors. The effect of CsA and TPA on virus induction in the B95-8 cell line would suggest either an alternative virus activation pathway, or the mediation of signals similar to IL-6, but at a stage after the binding of IL-6 to its receptor.
Fig 7. Induction of IL-6 by TPA, ionomycin, and TPA + ionomycin in EBV-immortalized or normal B lymphocytes. Three EBV-immortalized cell lines, each expressing low, intermediate, or high levels of IL-6, as well as P3HR-1, B95-8 and peripheral blood B cells, were cultured for 72 hours at $1 \times 10^6$ cells per milliliter in medium containing TPA (5 ng/mL) or ionomycin (1.25 µg/mL). Culture supernatants were analyzed for IL-6 activity and expressed as the average IL-6 U/mL ± SEM. Neither TPA nor ionomycin demonstrated 7TD1 growth activity (J.E.T., unpublished data, June 1994).

Control.

DISCUSSION

CsA treatment appears to be associated with a high incidence of lymphoproliferative disorders. Several lines of evidence indicate that CsA treatment is associated with an increase in B-cell activation in vivo. CsA-treated patients exhibit high levels of serum immunoglobulin, reversible LPD, and fatal B-cell lymphoma (with over 80% mortality if untreated). The etiologic factors involved in polyclonal B-cell activation and causes for the emergence of monoclonal and oligoclonal B-cell tumors in organ transplant recipients are presently unknown but probably arise from a complex interplay of multiple factors. In healthy individuals, EBV-transformed B cells are believed to be controlled by EBV-specific cytotoxic T cells, as well as by natural killer cells.

Results from the present study suggest that in addition to the known inhibitory actions of CsA on T-cell-dependent immune responses, CsA may also promote both the induction of the EBV growth factor, IL-6, in T cells and monocytes, and the activation of the EBV lytic-cycle in EBV-harboring cells. This ultimately could increase the number of EBV B-cell infections, as well as promote immortalization of B cells through increased paracrine IL-6, and thus potentially increase the opportunity for EBV B-cell lymphomas in immunosuppressed transplant patients.

The role of lytic virus infection in EBV LPD is still controversial. Patients undergoing immunosuppressive treatment may exhibit elevated levels of EA and VCA antibodies within the first few months. In one study the elevated serum levels of anti-EBV EA antibody appeared to precede the occurrence of an EBV lymphoma. Some have postulated that immunosuppressive therapy may simply deregulate control of anti-EBV Ig-expressing B cells, resulting in elevated serum titers of anti-VCA and anti-EA antibodies, without increasing virus production. Analysis of EBV-immortalized B cells cultured from organ transplant tumors showed expression of exclusively EBV-latent antigens. On the other hand, other studies suggest that immunosuppressive therapy may increase reactivation of the virus, particularly within the oropharynx. Oral administration of acyclovir or ganciclovir, drugs shown to inhibit viral DNA replication, have been found to be efficacious in drastically reversing EBV B-cell lymphomas. Finally, recent studies of tumor tissue have shown evidence of EBV lytic antigens as well as virion DNA. This difference among the various studies that found either EBV latent or lytic antigens could possibly be due to differences in culture conditions. Studies by Chen et al found that cells that were tested immediately upon explant exhibited high levels of EBV lytic-cycle antigens, whereas continued culture resulted in cells that displayed exclusively EBV latent-cycle antigens.

Our results indicate that CsA either directly or indirectly through IL-6 is capable of significantly inducing the lytic virus cycle in several EBV B-cell lines. These results suggest that in a patient undergoing CsA therapy, such treatment could potentially lead to lytic virus activation and shedding in the oropharynx, with potential to further infect B cells in the peripheral circulation. Furthermore, CsA treatment may induce virus at a potential tumor site for further B-cell infection and recruitment, potentially resulting in an EBV B-cell lymphoma.

In addition to stimulating virus production and EBV B-cell immortalization, CsA, in conjunction with virus, promoted IL-6 expression in T cells and monocytes. Although monocytes have not previously been demonstrated to contain significant levels of the EBV receptor, EBV and EBV-immortalized B cells have been shown to activate monocytes, and in rare cases, EBV may actually infect monocytes. Our results indicate that viral activation of monocytes is in large part mediated through the virion glycoproteins gp350 and gp220, as the neutralizing antibody 72A1, which has previously been shown to block virus binding, inhibited EBV induction of IL-6. Given the high carbohydrate content of the EBV ligands gp350 and gp220 (50% to 70% of the total molecular weight), it remains to be determined whether gp350 and gp220 interact specifically with CD21, or act similarly to other lectins to nonspecifically induce IL-6 production in monocytes.

Our results also demonstrate for the first time that IL-6 can promote the induction of the EBV lytic-cycle in some B cells (Table 1 and Fig 6). IL-6, which is an important B-cell differentiation factor, has been directly implicated in the pathogenesis of several human diseases, including Kaposi sarcoma, multiple myeloma, and Castleman’s disease. Additionally, IL-6 can promote EBV B-cell lymphoma formation by inhibiting natural killer cell activity in immunocompromised hosts. Several recent studies found that immunosuppressed, HIV-positive individuals, or posttransplant patients with increased IL-6 serum levels were predisposed to developing either EBV-positive lymphomas, as is the case for HIV-positive individuals, or posttransplant LPD, as exemplified in immunosuppressed graft recipients. In addition to acting as a paracrine or autocrine growth factor for EBV B cells, IL-6 could play an additional immunoinhibitory role(s) in the pathogenesis of
these EBV-related LPD. Our data suggest that if IL-6 is expressed at high levels in organ transplant patients due to CsA, such as within the microenvironment of an EBV B cell/T cell or an EBV B cell/macrophage interaction, the increased expression of IL-6 in conjunction with CsA could (1) potentiate B-cell immortalization through the action of IL-6 as a paracrine growth factor; (2) promote the virus lytic cycle in a manner similar to other known B-cell differentiation/virus-inducing agents, such as TPA and anti-Ig; or (3) act to limit cytotoxic functions and thus promote the outgrowth of B-cell lymphomas (Fig 8).12

A better understanding of the interactive role(s) CsA, IL-6, and EBV play in the promotion and propagation of NHL in immunosuppressed patients may provide a rational basis for novel therapeutic interventions and lymphoma prevention.

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