Rapamycin Inhibits the Interleukin 10 Signal Transduction Pathway and the Growth of Epstein Barr Virus B-cell Lymphomas

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ABSTRACT
EBV-infected B-cell lymphomas are a potentially life-threatening complication in bone marrow and solid organ transplant recipients. Immunosuppressive drugs required to prevent allograft rejection also impair anti-EBV T-cell immunity, thereby increasing the risk of EBV-associated disease. Here we demonstrate that the immunosuppressant rapamycin (RAPA) has a strong antiproliferative effect in vitro on B-cell lines derived from organ transplant recipients with EBV-associated posttransplant lymphoproliferative disorder (PTLD). Furthermore, RAPA significantly inhibits or delays the growth of solid tumors established from EBV-infected B-cell lines in a xenogeneic mouse model of PTLD. RAPA acts via cell cycle arrest, induction of apoptosis, and, most importantly, inhibition of interleukin 10 secretion, a necessary autocrine growth factor. The reduced interleukin 10 production is accompanied by corresponding decreases in the constitutive activation of the growth-promoting transcription factors signal transducer and activator of transcription 1 and 3. Thus, RAPA can limit B-cell lymphoma growth while simultaneously providing immunosuppression to prevent graft rejection in patients who are otherwise at risk for EBV-associated PTLD. Moreover, these findings may have application to other EBV-associated malignancies.

INTRODUCTION
EBV is a B lymphotropic herpesvirus associated with multiple human malignancies including nasopharyngeal carcinoma, Burkitt lymphoma, and Hodgkin’s lymphoma (1, 2). EBV is also the causative agent of the B-cell lymphomas in immunocompromised and iatrogenically immunosuppressed individuals (3). Solid organ and bone marrow transplant recipients are at increased risk for EBV-related malignancies because the immunosuppressive drugs required for prevention of graft rejection also impair host anti-EBV T-cell immunity.

In healthy individuals, EBV is maintained for the lifetime of the host as an episome in a subset of resting, peripheral memory B cells that express at most two virally encoded genes, EBNA1 and LMP2A (4). The limited expression of viral antigens in infected cells contributes to viral persistence through immune evasion. Intermittent reactivation of the virus in infected cells can give rise to activated B-cell blasts. However, the accompanying expression of the viral latent gene program is sufficient for detection and elimination by EBV-specific CTLs, thereby preventing lymphoproliferation in immunocompetent hosts.

EBV infection of B lymphocytes in vitro leads to immortalization and the establishment of long-term LCLs. The phenotype and viral gene expression profile of LCLs recapitulates that of the lymphoblasts that arise in vivo during primary infection, as well as the B-cell lymphomas associated with PTLD (5). Proliferation of EBV+ B lymphoblasts is governed by a complex interplay of viral and cellular growth and survival signals. For example, the oncogenic viral membrane protein LMP1 acts as a constitutively active, ligand-independent analogue of the B-cell surface molecule CD40 (6, 7) providing potent survival and proliferative signals.

Several lines of evidence indicate that autocrine cytokine pathways involving IL-6 and IL-10 are essential for the growth of EBV+ B cells. Neutralizing antibodies to IL-6 inhibit in vitro proliferation of EBV+ B-cell lines (8, 9) and tumor growth in SCID mice inoculated with EBV+ B-cell lines from patients with PTLD (10) or with lymphocytes from normal, seropositive donors (11). Similarly, we and others have shown that blockade of an autocrine IL-10 loop significantly inhibits proliferation of EBV+ B-cell lines derived from patients with PTLD (12) and AIDS-related B-cell lymphoma (13). We also showed that STAT1, STAT3, Jak1, Jak2, Jak3, and Tyk2 are constitutively tyrosine phosphorylated in EBV+ B-cell lymphomas, consistent with autocrine IL-10 signaling (14). Constitutive activation of the Jak/STAT pathway has been associated with multiple human malignancies including EBV-related Burkitt lymphoma (15) and multiple myeloma (16) and may contribute to uncontrolled growth, oncogenic transformation, or resistance to apoptosis (17).

RAPA is a microbial macrolide with potent immunosuppressive activity and proven clinical efficacy in the prevention of organ transplant rejection (18). RAPA has also shown promise as an anticancer drug (19). In lymphoid cells, RAPA inhibits cytokine-induced proliferation and causes arrest in the G1 phase of the cell cycle (20). RAPA interacts with the intracellular protein FK506-binding protein 12, forming a protein-drug complex that binds with high affinity to the mTOR (also known as FRAP1), a key regulatory kinase. Formation of this complex blocks mTOR function, thereby affecting multiple downstream signaling pathways required for protein synthesis and cell cycle progression.

Here we show that RAPA acts to suppress the in vitro and in vivo growth of EBV-infected B cells from patients with PTLD through a novel mechanism. RAPA inhibits production of the autocrine growth factor IL-10, thereby preventing tyrosine phosphorylation and formation of activated DNA-binding dimers of STAT1 and STAT3. In addition, RAPA induces cell cycle arrest and promotes apoptosis of EBV-infected B-cell lymphomas. Thus, RAPA is unique among the primary immunosuppressants used in clinical transplantation in that it may confer the dual advantage of preventing graft rejection while mitigating the development of EBV+ B-cell lymphomas.

MATERIALS AND METHODS

Antibodies and Reagents. Unless otherwise specified, all reagents were obtained from Sigma (St. Louis, MO). The following antibodies were used: antihuman CD20 (clone L26) and anti-Ki-67 (clone MIB-1) mAbs (DAKO, Carpinteria, CA); rabbit antihuman STAT1α (C-24) and rabbit antihuman STAT3 (H-190 (Santa Cruz Biotechnology, Santa Cruz, CA)); anti-P-Tyr-STAT1 (Tyr207); STIP-11A5) mAb (Zymed Laboratories, San Francisco, CA);
RAPAMYCIN AND EBV B-CELL LYMPHOMAS

rabbit antihuman P-Tyr-STAT3 (Tyr327) and rabbit antihuman P-Ser-STAT3 [Ser727] (Cell Signaling Technology, Beverly, MA); and anti-Jak1 and anti-
Tyr2 mAbs (BD Transduction Laboratories, San Diego, CA). RAPA (Rapamune; oral solution) and FK506 (Prograf; i.v. injection) used in the in vitro experiments were obtained from the Stanford University Medical Center Pharmacy. RAPA used in the in vivo experiments was obtained from Wyeth-Ayerst (Princeton, NJ).

Cell Lines. B LCLs were established by spontaneous outgrowth from the peripheral blood of liver transplant recipients (JB7 and FM4) or lymph node biopsy of a kidney transplant recipient (AB5) with EBV-related PTLD as described previously (12, 14). Briefly, the cell lines were generated by culturing isolated mononuclear cells from the blood or single cell suspensions from the lymph node biopsy in RPMI 1640 (Mediatech, Herndon, VA) with 10% FCS (Hyclone, Logan, UT), 100 µg/ml streptomycin and 100 units/ml penicillin (Life Technologies, Rockville, MD) at 1 × 10⁶ cells/ml in flat-bottomed 96-well plates. After 2 weeks, the surviving cells were expanded and maintained in RPMI 1640 supplemented with 10% FCS (Mediatech) and 100 µg/ml streptomycin and 100 units/ml penicillin. Because the cell lines were established in the absence of growth factors, mitogens, CSAs, or exogenous virus, they have been termed SLCLs. The SLCLs are EBV-infected as determined by PCR and Southern blot detection of the viral genome and express the human B-cell markers CD19, CD21, and CD23 (12, 14).

The EBV-infected Daudi Burkitt lymphoma and SKW6.4 B cell lines and the K562 human erythroleukemia cell line were obtained from the American Type Culture Collection (Manassas, VA) and cultured as described above. The JBush LCL was generated by in vitro EBV infection of normal human B cells.

In Vitro Drug Studies on Cell Growth and Cell Cycle Analysis. For proliferation experiments, cells were plated in triplicate in a 96-well plate at 2,000 cells/well. Cells were incubated at 37°C for 10 min for CD20 and P-Tyr-STAT3 and in Tris buffer [5 mM (pH 10.0), 20 mM] for Ki-67. Endogenous peroxidase was blocked with precoating with 1% hydrogen peroxide in PBS. Detection of bound primary antibodies was performed using the EnVision + System (DAKO), with a modified biotin-

histochemical staining of Tumor Tissue. Mouse tumor tissue was harvested at the time of sacrifice, fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned at 0.4-μm intervals. H&E-stained sections were examined concurrently with the patients’ original biopsies to confirm the initial diagnoses and to compare the histological features. The lymphoid neoplasms were classified as diffuse large B-cell lymphomas according to the WHO classification system (21).

Serial sections of 4 µm were cut from individual paraffin blocks, deparaf-
finized in xylene, and hydrated in a graded series of alcohol. Primary antibo-
dies were directed against CD20, Ki-67, and P-Tyr-STAT3. Antigen retrieval by microwave pretreatment was performed in citric acid buffer [10 mM (pH 6.0), 10 min] for CD20 and P-Tyr-STAT3 and in Tris buffer [5 mM (pH 10.0), 20 min] for Ki-67. Endogenous peroxidase was blocked by preincubation with 1% hydrogen peroxide in PBS. Detection of bound primary antibodies was performed using the EnVision + System (DAKO), with a modified biotin-


Statistical Analysis. Statistical analyses of tumor volume and IL-10 levels were performed using Student’s t test, and Ps of <0.05 were considered statistically significant.

RESULTS

RAPA Inhibits Growth of SLCLs in Vitro. We have shown previously that the calcineurin inhibitor immunosuppressants FK506 and CsA augment the growth of the SLCLs in vitro (23). To examine the effects of RAPA on SLCL growth, three PTLD-derived lymphoma lines, AB5, JB7, and MF4, along with Daudi, a Burkitt lymphoma cell line, JBush, an in vitro EBV-transformed LCL, and KS62, a human erythroleukemia cell line, were cultured with increasing doses of RAPA. Fig. IA shows that proliferation of the SLCLs, as well as Daudi and JBush, is markedly inhibited by RAPA in a dose-dependent manner. In contrast, RAPA has no effect on proliferation of KS62.

To determine whether this decrease in proliferation was due to cell growth arrest or apoptosis, cell cycle analysis was performed. RAPA causes an increase in the proportion of spontaneous lymphoblastoid cells in the G1 phase compared with the proportion of cells in S and G2/M, indicating an arrest in the G1 to S transition (Fig. 1B). FK506 blocks the ability of RAPA to induce G1 arrest in the SLCLs in a
dose-dependent manner. This blocking effect is likely due to competition between RAPA and FK506 for available cellular FK506-binding protein 12, the common intracellular target protein necessary for both drugs to form their active complexes. Simultaneous addition of FK506 also prevents the decrease in proliferation caused by RAPA (data not shown).

To examine the effect of RAPA on apoptosis, the proportion of cells with sub-G1 DNA content was also determined. RAPA induces a small but reproducible increase in the number of apoptotic cells (Fig. 1C). As described above, this effect is also blocked by concurrent addition of FK506. These data indicate that RAPA can inhibit cellular growth of PTLD-associated EBV+ B-cell lines through induction of cell cycle arrest and apoptosis.

Establishment of a Xenogeneic SCID Mouse Model of PTLD.

In agreement with previous reports (24, 25), SLCLs form tumors in SCID mice. SCID mice received s.c. injection with 7.5 × 10⁶ AB5 or JB7 cells and were treated for 8 weeks, beginning on the day of tumor inoculation, with either 1.5 mg/kg/day RAPA or carboxymethylcellulose vehicle. Control mice (n = 10) injected with JB7 cells developed solid tumors in the second to third week after tumor inoculation (Fig. 3A). By 6 weeks postinjection, 10 of 10 animals had visible s.c. tumors, and by 8 weeks, the average tumor volume in the control group was 9376 ± 1785 mm³ (mean ± SE). In sharp contrast, none of the mice in the experimental group (n = 10) had palpable tumors during the 8-week course of RAPA treatment (Fig. 3C). One animal subsequently developed a small, visible tumor (94 mm³) during the ninth week, and a second animal had a small tumor (50 mm³) at the site of injection that was discovered at autopsy.

Seven of the eight AB5-injected control mice developed solid tumors within 15 days of tumor injection (Fig. 3B). By 8 weeks after tumor injection the average tumor volume in the control group was 4421 ± 1277 mm³. In general, tumors in the AB5 control group were smaller than tumors in the JB7 control group. In contrast to the RAPA-treated JB7 mice, tumors developed in all RAPA-treated AB5 mice (Fig. 3D). However, tumor growth in the RAPA-treated AB5 animals was notably delayed, and the average tumor volume of the RAPA-treated group was significantly smaller (P < 0.05) than the average tumor volume in the AB5 control group between 2 and 7 weeks postinjection (Table 1). The three mice in the RAPA-treated group with the smallest tumors were removed from treatment 8 weeks after injection (Fig. 3D, inset). Cessation of RAPA treatment resulted in a dramatic increase in the average tumor volume (from 579 ± 95
control group was 125 mice. Whereas the average concentration of human IL-10 in the JB7 levels of human IL-10 were measured in control and RAPA-treated media alone. Note that the cells were counted after drug treatment, and the quantity of IL-10 was normalized to the number of viable cells. The data indicate that the decrease in IL-10 secretion results from a direct effect of RAPA on IL-10 expression and cannot be attributed to fewer cells in the RAPA-treated cultures caused by the antiproliferative effect of the drug. Concurrent addition of FK506 attributed to fewer cells in the RAPA-treated cultures caused by the antiproliferative effect of the drug. Concurrent addition of FK506.

RAPA Blocks IL-10 Secretion In Vitro and In Vivo. Our laboratory has demonstrated previously that SLCLs secrete and utilize IL-10 as an autocrine growth factor (12). To determine whether RAPA has an effect on IL-10 production by the SLCLs, ELISAs were performed on supernatants derived from cells cultured without and with RAPA. Daudi cells produce minimal IL-10 (26) and were not included in the experiment. Fig. 4A shows that RAPA causes a dose-dependent inhibition of IL-10 production by the SLCLs of over 75% for the three cell lines tested, compared with cells grown in media alone. Note that the cells were counted after drug treatment, and the quantity of IL-10 was normalized to the number of viable cells. The data indicate that the decrease in IL-10 secretion results from a direct effect of RAPA on IL-10 expression and cannot be attributed to fewer cells in the RAPA-treated cultures caused by the antiproliferative effect of the drug. Concurrent addition of FK506 restores IL-10 secretion by the SLCLs (data not shown).

To determine the effect of RAPA on IL-10 secretion in vivo, serum levels of human IL-10 were measured in control and RAPA-treated mice. Whereas the average concentration of human IL-10 in the JB7 control group was 125 ± 12 pg/ml at 8 weeks postinjection (Fig. 4B), almost no human IL-10 was detected in the sera of the JB7 RAPA-treated group (Fig. 4D).

In contrast to the RAPA-treated JB7 mice, RAPA-treated AB5 mice did have detectable levels of IL-10 consistent with tumor growth (Fig. 4E). However, at 4 weeks postinjection, the RAPA-treated AB5 group had significantly lower amounts of IL-10 than the control group (P = 0.002; Fig. 4, C and E). By 9 weeks postinjection, the average concentration of circulating human IL-10 in the treated group was 174 ± 51 pg/ml, compared with 1436 ± 511 pg/ml in the control group (P = 0.01). This demonstrates a direct correlation between tumor volume and IL-10 levels. For example, the two animals with minimal or no tumor growth in the AB5 control group (Fig. 3B) are the same two animals that had minimal or no detectable IL-10 (Fig. 4C). In the in vitro studies, we could attribute the diminished IL-10 levels in supernatants from RAPA-treated cells to a direct effect of the drug on IL-10 synthesis. However, in the in vivo experiments, the reduction in circulating IL-10 most likely reflects the smaller tumor burden in RAPA-treated mice, in addition to a direct effect on the production of IL-10.

RAPAMYCIN AND EBV B-CELL LYMPHOMAS

Fig. 2. Primary tumor and SLCL/SCID tumor histology. H&E-, CD20-, and Ki-67-stained paraffin sections of tonsil (JB7 primary tumor) and right axillary lymph node (AB5 primary tumor) with diffuse large cell lymphomas are shown. The H&E, CD20, and Ki-67 stains of representative tumors derived from SCID mice that received injection with JB7 and AB5 SLCL are shown in parallel for comparison. Immunohistochemical stain for CD20 demonstrates strong membrane reactivity compatible with B-cell lineage lymphomas. Immunohistochemical stain for Ki-67 shows nuclear reactivity in 60–80% of the lymphoma cells, indicating a high growth fraction.

STAT3 Is Constitutively Activated in Tumors from Patients with PTLD and in Tumors Established in SCID Mice. Recently, we demonstrated that the SLCLs express constitutively activated proteins of the Jak/STAT signal transduction pathway, likely resulting from autocrine IL-10 stimulation (14). In particular, STAT1 and STAT3 transcription factors remain tyrosine phosphorylated, forming the corresponding DNA-binding homo- and heterodimers. To determine whether constitutive STAT activation is a feature of EBV+ B-cell lymphomas in vivo, we performed immunohistochemical analysis using antibodies to P-Tyr-STAT3 on the original patient tumors and tumors from SCID mice that received injection with JB7 and AB5.

Staining for P-Tyr-STAT3 shows nuclear and cytoplasmic localization within lymphoma cells from both the patients and tumor-bearing SCID mice (Fig. 5). There is no appreciable difference by immunohistochemistry in the number of cells, intensity, or localization of the staining between primary patient tumors and the corresponding lymphomas established in SCID mice. These results indicate that STAT3 is constitutively activated in PTLD lymphomas in vivo.

RAPA Decreases Constitutive Jak/STAT Activation in the SLCLs. Because RAPA decreases IL-10 secretion by the SLCLs, and because constitutive STAT activation is associated with PTLD B-cell
lymphomas, we examined the effect of RAPA on the activation states of STAT1 and STAT3 by EMSA. Fig. 6 (top panel) demonstrates that RAPA decreases the quantity of activated STAT1 and STAT3 homo- and heterodimers present in the SLCLs. FK506 reverses this effect, as shown in the JB7 cell line (Fig. 6, bottom panel).

The formation of activated STAT dimers is the result of tyrosine phosphorylation and cross-interaction with the SH2 domains. To verify that the decrease in STAT dimers is due to an effect on the activation states of the proteins and not a decrease in the quantity of protein, both STAT1 and STAT3 were precipitated from lysates of untreated and RAPA-treated cells (Fig. 7). Blots were probed with P-Tyr-specific anti-STAT antibodies and then reprobed with the precipitating antibodies. Spot densitometry was performed to compare the amount of phosphorylated protein relative to the amount of total protein. RAPA inhibited tyrosine phosphorylation of STAT1 in the AB5 and JB7 cell lines by 28.8% and 55.1%, respectively, whereas no change was seen in STAT1 tyrosine phosphorylation in the MF4 cell line (Fig. 7A). A decrease in STAT3 tyrosine phosphorylation was observed in all SLCLs, with a reduction of 30.4% for AB5, 47.9% for JB7, and 27.2% for MF4 (Fig. 7B). The STAT1 tyrosine phosphorylation in the Daudi cell line was also decreased, as was the minimal amount of tyrosine-phosphorylated STAT3 in these cells.

Depending on the cellular context, serine phosphorylation may also be required for maximal STAT activation (27). In marked contrast to tyrosine phosphorylation, serine phosphorylation of STAT3 in the AB5, JB7, and MF4 cell lines was not affected by RAPA (Fig. 7C). Only Daudi cells showed a significant decrease in STAT3 serine phosphorylation (32.7%).

To further verify that the decrease in P-Tyr STAT proteins was not due to a decrease in the expression of proteins involved in the IL-10 signaling pathway, immunoblots were performed. Equal quantities of whole cell lysates from treated and untreated cells were separated and blotted for Jak1, Tyk2, STAT1, and STAT3. As shown in Fig. 7D, RAPA does not affect the expression of these proteins.

In summary, these data further support the idea that RAPA not only inhibits cell cycle progression and induces apoptosis but also acts by inhibiting IL-10 production and decreasing the tyrosine phosphorylation of the STAT proteins. This effect is specific to P-Tyr-STAT because RAPA does not inhibit serine phosphorylation of STAT3 or the overall expression of the Jak/STAT proteins in the IL-10 signaling pathway.
DISCUSSION

EBV+ B-cell lymphomas are a serious and potentially fatal complication of solid organ and bone marrow transplantation. The calcineurin inhibitors, CsA and FK506, increase the risk of EBV-related disease because they nonspecifically inhibit T lymphocytes, including EBV-specific CTLs responsible for controlling the expansion of EBV-infected B cells. Treatment options for PTLD are limited, and the prognosis is poor when traditional approaches such as chemotherapy, surgical resection, antiviral medications, or radiation are used (28). The first treatment strategy is usually to reduce or halt immunosuppression to allow the host’s immune system to recover and eliminate virally infected cells. However, this approach is often not effective and can precipitate graft rejection.

Here we demonstrate that the immunosuppressive drug RAPA directly inhibits the growth of EBV+ B-cell lymphomas at doses that are therapeutically effective for prevention of graft rejection (18). This observation has significance in the clinical management of transplant patients as well as the prevention and treatment of PTLD. Moreover, our findings indicate that RAPA targets a mTOR-dependent IL-10 autocrine growth pathway, resulting in diminished IL-10 production and inhibition of constitutive tyrosine phosphorylation of STAT1 and STAT3. Autocrine cytokine pathways are an important mechanism of tumorigenesis and tumor progression in lymphoid malignancies, and disruption of these pathways can have profound effects on tumor growth.

It is plausible that RAPA interferes with the IL-10 autocrine growth axis at multiple sites. Our data clearly demonstrate that RAPA inhibits IL-10 production leading to diminished signaling through the IL-10 receptor. Previous reports have shown that RAPA also inhibits IL-10 production by mitogen-activated T cells (30) and peripheral blood mononuclear cells (31); however, there are no reports showing RAPA inhibition of B-cell derived IL-10 or inhibition of autocrine cytokine pathways.

Our data show that RAPA does not alter the levels of the endogenous Jak/STAT proteins. On the other hand, we cannot rule out the possibility that RAPA also acts downstream of the IL-10 receptor to
inhibit the constitutive phosphorylation of STAT1 and STAT3, independently of its effect on IL-10 production. Along these lines, it has been shown that tyrosine phosphorylation of STAT3 induced by anti-immunoglobulin in murine B cells is RAPA sensitive and is not due to inhibition of growth factor production (32). Tyrosine phosphorylation of STAT is critical for dimerization, translocation to the nucleus, and binding to STAT-responsive elements. Depending on the stimulus and the cellular context, phosphorylation on Ser727 of

![Image](44x404 to 560x748)

**Fig. 5.** STAT3 is tyrosine phosphorylated in primary tumors and SLCL/SCID tumors. Immunohistochemical stain for P-Tyr-STAT3 shows nuclear and cytoplasmic staining in the primary patient tumors and in the corresponding tumors derived from SCID mice.

![Image](262x61 to 560x305)

**Fig. 6.** RAPA inhibits constitutive STAT dimer formation in the SLCLs. Cells were treated for 3 days in the absence or presence of 10 ng/ml RAPA (top panel) without and with 100 ng/ml FK506 (bottom panel). Extracts were prepared, and 20 μg of protein were incubated with a 32P-labeled hSIE probe, in the presence or absence of a 100-fold molar excess of unlabeled probe (+100× cold oligo). Complexes were separated on a 4% nondenaturing polyacrylamide gel, which was then exposed to film for 18 h at −70°C. Activated STAT1 and STAT3 homo- and heterodimers are indicated.
STAT3 may also be essential for maximal STAT3 transactivation (27). At least in the case of ciliary neurotrophic factor-stimulated neuroblastoma cells, mTOR can phosphorylate Ser727 of STAT3 (33). Although our data indicate that STAT3 Ser727 is constitutively phosphorylated in the EBV+ B-cell lines, RAPA does not effect the serine phosphorylation status of STAT3, suggesting that mTOR does not play a role in serine phosphorylation in these cells.

At present, our data most directly support the idea that RAPA inhibits IL-10-mediated activation of the Jak/STAT signal transduction pathway, resulting in suppression of tumor growth. How activation of the transcription factors STAT1 and STAT3 may drive cellular growth is not known nor are the relevant target genes known. Nevertheless, constitutive STAT activation is a prominent feature of many growth is not known nor are the relevant target genes known. Nevertheless, constitutive STAT activation is a prominent feature of many

In summary, we report that RAPA inhibits a mTOR-dependent autocrine growth pathway in EBV+ B-cell lymphomas that is crucial for tumor growth. The data presented here have significant implications for the management of transplant recipients at risk for EBV-related disease because they suggest an approach to simultaneously maintain adequate immunosuppression while deterring the development of EBV+ B-cell lymphomas. Furthermore, these findings may have significance for the treatment of other EBV-related malignancies.

Taken together with our findings in a xenogeneic model of PTLD, it appears that the mechanism of RAPA’s antitumor effect can vary depending on the tumor type. Thus, RAPA can influence a broad range of biological processes involved in tumor progression including tumorigenesis, growth, survival, and metastasis.

Our in vitro experiments showed that RAPA also caused accumulation of cells in the G1 phase, leading to cell cycle arrest in the G1 to S transition, and a minor but reproducible induction of apoptosis, both of which were reversed by FK506. Each of these effects by RAPA have been observed in a variety of normal and transformed cells (20). The antitumor activity of RAD, a RAPA derivative, on in vitro infected EBV+ B-cell lines was also attributed to cell cycle arrest and apoptosis (38).

Whereas RAPA was able to suppress tumor growth of the AB5 SLCL, it dramatically inhibited tumor establishment of the JB7 SLCL in a xenogeneic mouse model of PTLD. The reason for the variable sensitivity to RAPA by the two EBV+ B-cell lymphomas is unknown. However, it is interesting to note that the differences in the effect of RAPA on tumor growth in vivo correlate with the relative in vitro sensitivities of the two cell lines to RAPA. Accordingly, there was greater inhibition of proliferation, and greater inhibition of STAT1 and STAT3 activation, in the JB7 cell line as compared with the AB5 cell line.

In summary, we report that RAPA inhibits a mTOR-dependent autocrine growth pathway in EBV+ B-cell lymphomas that is crucial for tumor growth. The data presented here have significant implications for the management of transplant recipients at risk for EBV-related disease because they suggest an approach to simultaneously maintain adequate immunosuppression while deterring the development of EBV+ B-cell lymphomas. Furthermore, these findings may have significance for the treatment of other EBV-related malignancies.
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References


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