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Licking latency with licorice

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Commentary

Numerous viruses cause latent infections in humans, and reactivation often results in pain and suffering. While vaccines for several of these viruses are available or currently being studied in clinical trials, and antiviral therapies have been successful in preventing or treating active infection, therapy to eradicate latent infection has lagged behind. A new study reported in this issue of the *JCI* shows that treatment of cells latently infected with Kaposi sarcoma–associated herpesvirus (KSHV) with glycyrrhizic acid, a component of licorice, reduces synthesis of a viral latency protein and induces apoptosis of infected cells. This finding suggests a novel way to interrupt latency.

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Licking latency with licorice

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Numerous viruses cause latent infections in humans, and reactivation often results in pain and suffering. While vaccines for several of these viruses are available or currently being studied in clinical trials, and antiviral therapies have been successful in preventing or treating active infection, therapy to eradicate latent infection has lagged behind. A new study reported in this issue of the *JCI* shows that treatment of cells latently infected with Kaposi sarcoma–associated herpesvirus (KSHV) with glycyrrhizic acid, a component of licorice, reduces synthesis of a viral latency protein and induces apoptosis of infected cells (see the related article beginning on page 642). This finding suggests a novel way to interrupt latency.

Licorice, derived from the root of *Glycyrrhiza glabra*, has been used for more than 4 millennia as a flavoring agent in foods, beverages, and tobacco (1). Licorice is also used as an alternative medicine for the treatment of gastric and duodenal ulcers, sore throat, bronchitis, cough, arthritis, adrenal insufficiency, and allergic diseases. The licorice root contains numerous compounds, including glycyrrhizic acid (GA). It is estimated that in the United States, 3.3 mg of GA is consumed per person daily. GA inhibits the replication of several viruses in vitro including herpesviruses, HIV, and the SARS coronavirus. When taken orally, GA is hydrolyzed to glycyrrhetic acid by bacteria in the gastrointestinal tract before GA can be absorbed. Therefore, in Asia, where GA is used for the treatment of chronic hepatitis B or C infection, the drug is infused intravenously to achieve the appropriate serum levels.

In this issue of the *JCI*, Curreli et al. (2) show that GA induces apoptosis of primary effusion lymphoma (PEL) cells that are transformed by Kaposi sarcoma–associated herpesvirus (KSHV). KSHV is the etiologic agent of Kaposi sarcoma, and the virus is present in lesions from patients with multicentric Castleman disease and PEL. The latter presents as a malignant effusion located in the pleural, peritoneal, or pericardial space; tumor cells can also infiltrate the adjacent tissues. The virus is latent in PEL cells, which express a very limited set of viral proteins. The median survival time after diagnosis for patients with PEL is 6 to 12 months with chemotherapy and radiation therapy; thus, newer approaches to therapy are needed.

Curreli et al. (2) found that GA downregulates synthesis of the KSHV latency-associated nuclear antigen 1 (LANA-1) (Figure 1). LANA-1 is expressed in all KSHV-infected cells, including PEL cells. This protein allows the viral genome to be maintained as an episome in latently infected cells. LANA-1 binds to p53, inhibiting p53-mediated apoptosis, and interacts with the retinoblastoma tumor–suppressor protein (Rb), which may prevent Rb-mediated cell cycle arrest. Curreli et al. found that downregulation of LANA-1 by GA was associated with an alteration in the mitochondrial membrane potential with translocation of apoptosis-inducing factor to the nucleus, DNA fragmentation, and apoptosis (2). In addition, cells treated with GA showed higher levels of phosphorylated (active) p53, which resulted in cell cycle arrest at the G1 checkpoint. GA upregulated expression of the KSHV cyclin protein (v-cyclin) but did not affect expression of the viral FLICE-inhibitory protein (vFLIP). V-cyclin binds to and activates cyclin-dependent kinase 6, which results in phosphorylation and inactivation of p53 and Rb. The increased level of v-cyclin in PEL cells treated with GA might also contribute to cell death, since overproduction of the protein has been reported to induce apoptosis.

Additional molecular approaches to killing latent KSHV–infected PEL cells

Other approaches have been considered for the treatment of PEL, based on KSHV gene expression in these tumors. Tumor cells

Nonstandard abbreviations used: EBV, Epstein-Barr virus; GA, glycyrrhizic acid; KSHV, Kaposi sarcoma–associated herpesvirus; IL-6R, IL-6 receptor; LANA-1, latency-associated nuclear antigen 1; PEL, primary effusion lymphoma; Rb, retinoblastoma tumor–suppressor protein; v-cyclin, KSHV cyclin protein; vFLIP, viral FLICE-inhibitory protein; vIL-6, viral IL-6.

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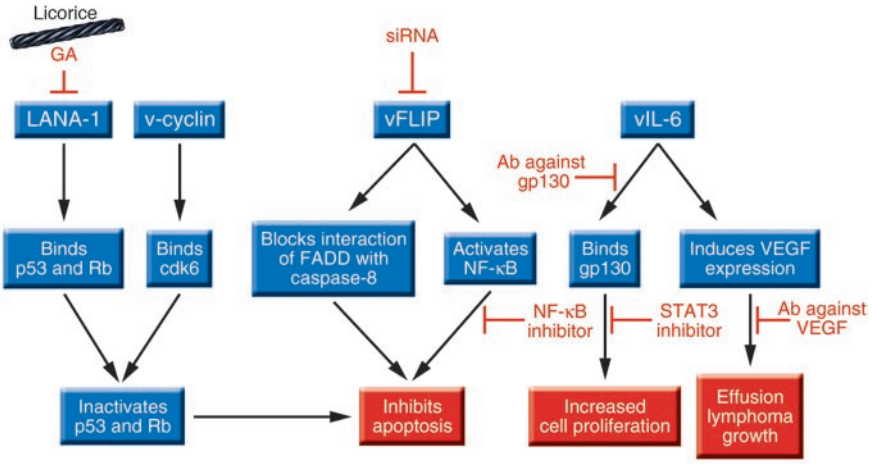

Figure 1
Mechanisms of action of KSHV latency proteins expressed in PEL cells and points of attack by inhibitor compounds. cdk6, cyclin-dependent kinase 6; FADD, Fas-associated death domain–containing protein; siRNA, small interfering RNA.

from patients with PEL produce several KSHV latency proteins, including LANA-1, v-cyclin, vFLIP, and viral IL-6 (vIL-6) (Figure 1) (3–5). vFLIP inhibits Fas-induced apoptosis and is responsible for most of the activation of NF-κB in PEL cells. Prevention of vFLIP production in PEL cells by transfection with small interfering RNA inhibited NF-κB activity and induced apoptosis of the cells (6, 7). In addition, treatment of PEL cells with a small molecule that inhibits NF-κB (Bay 11-7082) induced apoptosis of these cells (8).

PEL cells synthesize vIL-6 in vivo, and PEL cells secrete human IL-6 and IL-10 and vIL-6 in vitro. vIL-6 functions as an autocrine growth factor for PEL cells in vitro, and the protein interacts with the signal transduction protein gp130 but not with the human IL-6 receptor (IL-6R). The interaction of vIL-6 with gp130 leads to phosphorylation of STAT3, activation of the JAK-STAT pathway, and cell proliferation. Inhibition of STAT3 signaling by transfection of PEL cells with a dominant negative form of STAT3, or treatment of cells with a drug (AG490) that inhibits JAK2, resulted in decreased production of survivin and apoptosis of the cells (9). Other approaches have been used to inhibit the activity of IL-6. Antibody against gp130 or soluble IL-6R inhibited PEL cell growth in vitro (10). In another study, antibody against IL-6 inhibited growth of PEL cell tumors in SCID mice (11). Antibody against human IL-10, and to a lesser extent antibody against vIL-6, inhibited growth of PEL cells in vitro (12). vIL-6 induces expression of VEGF, which promotes angiogenesis. PEL cells secrete high levels of VEGF and have VEGF receptors on their surface (13). Administration of neutralizing antibody against VEGF prevented development of effusion lymphomas in irradiated SCID/beige mice injected intraperitoneally with PEL cells.

Targeted attack on latency of another human herpesvirus: Epstein-Barr virus

Several approaches have been used to interrupt latency of Epstein-Barr virus (EBV), the other human γ-herpesvirus, which like KSHV infects and transforms B cells. Hydroxyurea eliminated EBV episomes from EBV-positive Burkitt lymphoma cells and reduced their malignant phenotype in an animal model (14). Low-dose hydroxyurea reduced the tumor size of EBV-positive central nervous system lymphomas in 2 patients with HIV (15).

Treatment of B cells latently infected with EBV with arginine butyrate (16) or gemcitabine (17) followed by ganciclovir induced viral replication and expression of the viral thymidine kinase, resulting in phosphorylation of ganciclovir and cell death in vitro. Infusions of EBV-specific cytotoxic T cells have been used successfully to treat patients with EBV lymphoproliferative disease (18) or EBV-positive Hodgkin disease (19), both of which are due to tumor cells latently infected with virus. Such approaches might also be tried for the treatment of patients with KSHV-positive PEL.

Future considerations for GA therapy

While GA is effective for killing PEL cells in vitro, there are several caveats for the treatment of patients with PEL with GA. First, since GA is rapidly hydrolyzed to glycyrrhetic acid in the gastrointestinal tract, glycyrrhetic acid would need to be shown to be effective against PEL cells in vitro or GA would need to be administered intravenously. After intravenous administration of GA for treatment of hepatitis, serum levels of GA have been shown to range from 40 to 100 μg/ml (20), compared with the millimolar concentrations needed to induce apoptosis of PEL cells in vitro. Thus, the levels of GA required for efficacy in vitro might not be achievable in vivo. Second, Curreli et al. (2) found that the effects of GA on downregulating LANA expression in PEL cells were reversible for up to 3–4 days of treatment; thus, continuous and/or prolonged courses of therapy with GA might be needed. Third, therapeutic levels of GA might be toxic for normal cells and tissues. Curreli et al. found that the ED_{50} of GA for PEL cells was 2–3 mM; however, levels of 5–6 mM were toxic for uninfected cells. Thus the therapeutic index for the treatment of PEL is likely to be low. Finally, it is not clear whether the effects of GA on PEL cell lines in vitro would also occur in tumors in vivo. Since intraperitoneal inoculation of immunodeficient mice with PEL cells results in malignant ascites, intravenous infusions of GA could be tested for efficacy in this model.

While a compound present in licorice may seem like an unlikely candidate for the treatment of virus-associated cancers, it is important to remember that other traditional drugs have proved highly effective for some infectious diseases. Extracts of the wormwood plant, which is a traditional Chinese medication for treatment of febrile illnesses, contain artemisinin; derivatives of this compound have become first-line treatments for drug-resistant

malaria. Thus, derivatives of GA or other traditional medicines might be used in the future for treating human diseases caused by latent virus infections.

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Unlocking the DEAD-box: a key to cryptococcal virulence?

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The DEAD-box RNA helicases are enzymes involved in many critical aspects of RNA metabolism within both eukaryotic and prokaryotic organisms. Several studies have shown that these proteins may have important functions in mediating microbial pathogenesis. A new study in this issue of the *JCI* identifies the first DEAD-box RNA helicase in the pathogenic fungus *Cryptococcus neoformans* and proposes novel roles for this family of proteins in the development and progression of cryptococcosis (see the related article beginning on page 632).

Cryptococcosis is a chronic human disease caused by the ubiquitous environmental fungus *Cryptococcus neoformans*. The disease occurs after inhalation of yeast cells or basidiospores into the alveolar spaces and eventually progresses with the dissemination of *C. neoformans* to the central nervous system, causing meningoencephalitis (1). The majority of cryptococcosis cases have been reported in immunocompromised patients, such as subjects with AIDS or those undergoing transplantation, but certain varieties of *C. neoformans* do affect immunocompetent hosts (1). Current therapies cannot completely eradicate the chronic infection, which necessitates life-long treatment. Therefore, studies addressing the understanding of pathophysiological processes leading to the development of the disease are particularly important for the discovery of new therapeutic strategies. *C. neoformans* is a facultative intracellular pathogen with several well-established virulence factors, including growth at 37°C (temperature of the mammalian host), a large antiphagocytic polysaccharide capsule, and the laccase enzyme, which can produce melanin pigments from host-derived substrates. With the goal of identifying novel targets for drug development, current *Cryptococcus* research is focused on the signaling networks that regulate these virulence factors. A few virulence-related pathways have been identified in *C. neoformans*, including the Gα protein-cAMP-PKA and Ipc1-Pkc1 pathways (2, 3). In this issue of the *JCI*, Panepinto et al. (4) describe a new class of proteins and a novel signaling net-

Nonstandard abbreviations used: Not, negative on TATA-less; RNAi, RNA interference; SF, superfamily; Vad1, virulence-associated DEAD-box RNA helicase–encoding protein.

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