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Evidence that Amphotericin B Mediates Reactivation of Latent Epstein-Barr Virus in Hodgkin's Lymphoma Allowing Cytotoxicity by Acyclovir

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This brief communication focuses on aspects of a recent case report (Yonsei Med J 2005;46:425-30) on a full and sustained remission of Hodgkin's lymphoma (HL) after a single day of chemotherapy. A septic episode required stopping chemotherapy and starting amphotericin B and acyclovir. Remission evidence was seen within days of starting these. A review of research supporting the notion that amphotericin B can reactivate latent Epstein-Barr virus and thus allow acyclovir to kill infected HL cells is given. Experimental work is required to confirm or refute this possibility. If successful, amphotericin B and acyclovir treatment could be extended to other EBV-driven cancers such as Burkitt's lymphoma, nasopharyngeal carcinoma and the occasional EBV-related epithelial cancer of the breast, colon, prostate, and others.

Key Words: Acyclovir, amphotericin B, Coley's toxin, Epstein-Barr virus, ganciclovir, Hodgkin's lymphoma, remission, TNF-α

In the June 2005 issue of the Yonsei Medical Journal,[1] Bang et al. reported a 36-year-old male with Hodgkins lymphoma (HL) mixed cellularity type, stage IVB, who showed signs of regression after a single day of COPP-AV (cyclophosphamide, vincristine, procarbazine, prednisone- doxorubicin, vinblastine). IgM for the Epstein-Barr virus (EBV) was negative, indicating that this HL was not associated with recent infectious mononucleosis. One day after COPP-AV, on hospital day 16, dyspnea and X-ray evidence of bilateral pneumonia and Candida antigenemia were noted. Amphotericin B and acyclovir were started along with several antibacterial antibiotics. Candida antigenemia was indicative of immunosuppression, hence treatment initiation with acyclovir to treat or prevent concomitant cytomegalovirus (a Herpes virus). Acyclovir was switched to its homologue ganciclovir. Five days after amphotericin B and acyclovir/ganciclovir, A/G, treatment initiation, on hospital day 21, and after multiple antibiotics, a repeat chest CT scan showed resolving pneumonitis and smaller mediastinal and supraclavicular lymph nodes that had been enlarged on admission. After 2 months of hypoxic episodes, intubation, bouts with diabetes insipitus, pancreatitis, and peripheral neuropathy, on hospital day 90, a repeat CT scan showed slight evidence of lymphadenopathy, most areas having resolved. Nine months later and without further treatment, there was no evidence of HL. This patient remains well now, 4 years later.

The sustained remission is not only astounding itself, but also the rapidity at which it began. This case therefore requires careful and detailed consideration. Bang at al. conjectured that remission was a consequence of the septic episode, as it well might have been. Cancer remission after sepsis has a long history, which was reviewed for the centenary of William B. Coley in 1994.[2] In the late 1890's Coley saw remissions after iatrogenically induced erysipelas. He saw this only for sarcomas, but these included lymphomas. Bang et al. place their case in this category of cancer remission induced by sepsis-triggered, massive inflammatory mediator release and/or induction of immunological events leading to HL cytotoxi-

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city.

An alternative explanation expounded herein, is that the HL cells were infected with EBV. Amphotericin B has profound effects on lymphocytes. Derangements wrought by amphotericin B could have reactivated a latent EBV infection that entailed transcription of EBV thymidine kinase that phosphorylates A/G, creating toxic nucleoside analogues that killed EBV-infected and nearby cells.

HL is an unusual cancer for several reasons:[3]

1. Only 1% of the HL tumor mass is composed of malignant cells. The remaining 99% is composed of non-malignant lymphocytes, monocyte lineage cells, mast cells, eosinophils and fibroblasts. Such a lavish array of non-malignant cells is quite unique.
2. The malignant HL cell, long thought to be of cryptic origin, is now recognized to be of B lymphocyte origin, though morphologically deformed (several times larger, paler, less round, with prominent nucleolus, and often multinucleated). When a single nucleus is present it is termed a Hodgkin cell, when multinucleated, a Reed-Sternberg cell (together termed HRS cells).
3. One in 10^7 to 10^9 of resting B lymphocytes in 95% of adults worldwide harbor latent EBV, but approximately half of all HL cases have all HRS cells infected with latent EBV (60% in HL of mixed cellularity, as in Bang's case). This implies that 95% of all HL cases have HRS cells with some EBV-positive lymphocytes nearby in the 99% tumor mass composed of non-malignant lymphocytes and other cells.
4. Substantial numbers of non-malignant lymphocytes within the HL tumor mass are of the CD4+CD25+ immunosuppressive phenotype, contributing to immunosuppression seen in HL patients.[4]

Herpes simplex, Herpes varicella zoster, cytomegalovirus, the Herpes virus causing Kaposi's sarcoma, and EBV are all Herpes viruses and share similar life cycle patterns and attributes common to Herpes viruses: 1) initial transient infection of, and active replication in, an epithelium followed by 2) epigenomic residence in a second, non-epithelium cell type (lymphocytes for EBV and neurons for both H. simplex and H. zoster, for example). Then, 3) low level epigenomic replication occurs in the secondary cell type at the time of host cell division, assuring that both daughter cells are infected; other than this, infectious EBV are not produced or only minimally so. This state is latent infection. 4) Infrequent reactivation occurs where large numbers of complete viruses are produced. There is then full expression of EBV-coded proteins. This is lytic infection.

Acyclovir, famciclovir, ganciclovir, penciclovir and valacyclovir are homologues sharing similar anti-Herpes attributes. Ganciclovir is reviewed in ref. 5 as representative of the group.[5] Latent infected HRS cells do not express virally coded thymidine kinase, and therefore A/G has little cytotoxic effect on them until and unless active lytic infection can be triggered when large amounts of A/G are phosphorylated into cytotoxic nucleoside analogues.

Inducing lytic EBV reactivation in cancer cells that bear latent EBV infection in order to allow A/G to kill them is an old idea:[6]

1. Feng et al.[7] suggested therapy for EBV-positive tumors using intentional induction of the lytic form of EBV infection combined with ganciclovir treatment. "The addition of ganciclovir to either gemcitabine- or doxorubicin-containing chemotherapy regimens may enhance the therapeutic efficacy of these drugs for EBV-driven lymphoproliferative disease ...".
2. Feng et al.[8] also showed evidence that methotrexate can reactivate ganciclovir phosphorylation activity in EBV latent infection and suggested methotrexate in the treatment of EBV-driven lymphoproliferative malignancies as above.
3. Faller at al.[6] suggested using arginine butyrate to trigger lytic reactivation with ganciclovir to treat EBV-associated malignancies, and showed that it can do this.[9] The butyrate moiety is active in this regard. A butyrate drug is already on the market in many coun-

tries in the form of gamma- hydroxybutyrate (Oxybate). It is currently approved to treat narcolepsy but its use with A/G could be explored for EBV treatment.

4. Daibata et al. have shown[9] that dexamethasone can induce ganciclovir phosphorylation activity in EBV latently infected lymphoma cells *in vitro.*
5. Empirically, COPP-AV treatment of HL induces some latent infected B cells, either HRS cells themselves or EBV-positive B cell bystanders, to undergo EBV reactivation,[10] suggesting that A/G might augment the therapeutic effects of current chemotherapy for EBV-positive tumors.
6. Nasopharyngeal carcinoma is associated with EBV infection. When grown in nude mice, human explanted nasopharyngeal carcinoma both shows EBV reactivation and becomes susceptible to being killed by A/G homologues.[11]

Amphotericin B, used in humans for over fifty years, is still one of the most potent anti-fungal agents available. By preferential binding to ergosterol compared to cholesterol, amphotericin B preferentially kills fungi and single cell parasites like Leishmania spp. Amphotericin B disrupts lymphocytes' lipid-rich rafts and functions empirically as a polyclonal B cell activator, triggering immunoglobulin synthesis and maturation-stimuli known to trigger EBV reactivation. When used clinically to treat fungal infection, amphotericin B can cause massive inflammatory mediator release, in part by stimulating Toll-like receptor-2,[12,13] resulting in the activation of B lymphocytes (including I conjecture of the HRS cell itself).

Two related observations are of note here: 1) An adult T cell leukemia remitted after pulmonary and urinary tract infection.[14] Again, amphotericin B was used over a period of many months just prior to and during the remission. This adult T cell leukemia relapsed fatally as a lymphoma when amphotericin B was stopped. 2) Pseudorabies virus is a swine Herpes virus related to H. simplex (and quite unrelated to rabies virus). Augmentation of antiviral effects of acyclovir by amphotericin B was previously noted 22 years ago[15] in an *in vitro* system with pseudorabies, further linking amphotericin B to activation of Herpes thymidine kinase.

In conclusion, in Bang et al.'s patient, amphotericin B may have reactivated latent EBV in HRS cells, allowing A/G to kill them. Perhaps nearby nonmalignant EBV-positive lymphocytes had their latent EBV activated by amphotericin B and they generated enough cytotoxic A/G metabolites to kill the HRS clone. Components of COPP-AV have been shown to reactivate EBV lytic infection and may have done so, or may have contributed to reactivation intensity. Given the safety of amphotericin B and A/G compared to current cytotoxic treatments like COPP-AV or irradiation, A/G treatment of HL should be further explored and the current hypothesis tested.

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