<table>
<thead>
<tr>
<th>Name</th>
<th>Page(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aboulafia</td>
<td>87,90</td>
</tr>
<tr>
<td>Anderson</td>
<td>72</td>
</tr>
<tr>
<td>Astrow</td>
<td>11</td>
</tr>
<tr>
<td>Ayers</td>
<td>86</td>
</tr>
<tr>
<td>Biberfeld</td>
<td>7</td>
</tr>
<tr>
<td>Biggar</td>
<td>58</td>
</tr>
<tr>
<td>Boue</td>
<td>89</td>
</tr>
<tr>
<td>Broker</td>
<td>S2</td>
</tr>
<tr>
<td>Brown</td>
<td>60</td>
</tr>
<tr>
<td>Bryant</td>
<td>88</td>
</tr>
<tr>
<td>Butler</td>
<td>4</td>
</tr>
<tr>
<td>Casper</td>
<td>5</td>
</tr>
<tr>
<td>Casper</td>
<td>5,12</td>
</tr>
<tr>
<td>Chow</td>
<td>16</td>
</tr>
<tr>
<td>Crabb Breen</td>
<td>8</td>
</tr>
<tr>
<td>Dalla Favera</td>
<td>S13</td>
</tr>
<tr>
<td>de Sanjose</td>
<td>26</td>
</tr>
<tr>
<td>DeFalco</td>
<td>81</td>
</tr>
<tr>
<td>Dezube</td>
<td>64</td>
</tr>
<tr>
<td>Dittmer</td>
<td>14</td>
</tr>
<tr>
<td>Dorjsuren</td>
<td>52</td>
</tr>
<tr>
<td>Duell</td>
<td>42</td>
</tr>
<tr>
<td>Dupin</td>
<td>10</td>
</tr>
<tr>
<td>Dutta</td>
<td>18</td>
</tr>
<tr>
<td>Farrell</td>
<td>17</td>
</tr>
<tr>
<td>Ferfelia</td>
<td>80</td>
</tr>
<tr>
<td>Foreman</td>
<td>45</td>
</tr>
<tr>
<td>Grant</td>
<td>77</td>
</tr>
<tr>
<td>Graves Jones</td>
<td>56</td>
</tr>
<tr>
<td>Guasparri</td>
<td>15</td>
</tr>
<tr>
<td>Guimond</td>
<td>23</td>
</tr>
<tr>
<td>Hall</td>
<td>19</td>
</tr>
<tr>
<td>Hassan</td>
<td>22</td>
</tr>
<tr>
<td>Hayward</td>
<td>S11</td>
</tr>
<tr>
<td>Hermine</td>
<td>S15</td>
</tr>
<tr>
<td>Hessol</td>
<td>1</td>
</tr>
<tr>
<td>Hoffmann</td>
<td>30,31</td>
</tr>
<tr>
<td>Hollwitz</td>
<td>75</td>
</tr>
<tr>
<td>Janz</td>
<td>25</td>
</tr>
<tr>
<td>Ji</td>
<td>71</td>
</tr>
<tr>
<td>John</td>
<td>66</td>
</tr>
<tr>
<td>Kaplan</td>
<td>S17</td>
</tr>
<tr>
<td>Koon</td>
<td>20</td>
</tr>
<tr>
<td>Laney</td>
<td>3</td>
</tr>
<tr>
<td>Levine</td>
<td>21</td>
</tr>
<tr>
<td>Liao</td>
<td>46,47</td>
</tr>
<tr>
<td>Lieberman</td>
<td>24,37</td>
</tr>
<tr>
<td>Little</td>
<td>S16</td>
</tr>
<tr>
<td>Lowy</td>
<td>S3</td>
</tr>
<tr>
<td>Lubyova</td>
<td>13</td>
</tr>
<tr>
<td>Mbulaiteye</td>
<td>6</td>
</tr>
<tr>
<td>McDowell</td>
<td>43</td>
</tr>
<tr>
<td>McGhee</td>
<td>83</td>
</tr>
<tr>
<td>Melief</td>
<td>S5</td>
</tr>
<tr>
<td>Mesri</td>
<td>36</td>
</tr>
<tr>
<td>Montaner</td>
<td>35</td>
</tr>
<tr>
<td>Morgan</td>
<td>69</td>
</tr>
<tr>
<td>Muderspach</td>
<td>S6</td>
</tr>
<tr>
<td>Mullerat</td>
<td>78,79</td>
</tr>
<tr>
<td>Mutlu</td>
<td>53</td>
</tr>
<tr>
<td>Noonan</td>
<td>61</td>
</tr>
<tr>
<td>Orem</td>
<td>28</td>
</tr>
<tr>
<td>Orth</td>
<td>S9</td>
</tr>
<tr>
<td>Otieno</td>
<td>29</td>
</tr>
<tr>
<td>Pantanowitz</td>
<td>62</td>
</tr>
<tr>
<td>Panther</td>
<td>70</td>
</tr>
<tr>
<td>Pati</td>
<td>51</td>
</tr>
<tr>
<td>Phiri</td>
<td>57</td>
</tr>
<tr>
<td>Pozharskava</td>
<td>44</td>
</tr>
<tr>
<td>Prakash</td>
<td>54</td>
</tr>
<tr>
<td>Preston-Martin</td>
<td>2</td>
</tr>
<tr>
<td>Pumfery</td>
<td>34</td>
</tr>
<tr>
<td>Raab-Traub</td>
<td>S10</td>
</tr>
<tr>
<td>Ragin</td>
<td>65</td>
</tr>
<tr>
<td>Ramos</td>
<td>41</td>
</tr>
<tr>
<td>Rubin</td>
<td>91</td>
</tr>
<tr>
<td>Samaniego</td>
<td>39,40</td>
</tr>
<tr>
<td>Sandulyak</td>
<td>84</td>
</tr>
<tr>
<td>Sastry</td>
<td>68</td>
</tr>
<tr>
<td>Schlegel</td>
<td>S12</td>
</tr>
<tr>
<td>Sharp</td>
<td>S1</td>
</tr>
<tr>
<td>Shipp</td>
<td>S14</td>
</tr>
<tr>
<td>Simonelli</td>
<td>32</td>
</tr>
<tr>
<td>Song</td>
<td>76</td>
</tr>
<tr>
<td>Srinivasan</td>
<td>27</td>
</tr>
<tr>
<td>Strickler</td>
<td>S6</td>
</tr>
<tr>
<td>Sugaya</td>
<td>55</td>
</tr>
<tr>
<td>Tang</td>
<td>38</td>
</tr>
<tr>
<td>Tary-Laehmann</td>
<td>82</td>
</tr>
<tr>
<td>Taylor</td>
<td>73</td>
</tr>
<tr>
<td>Teo</td>
<td>59</td>
</tr>
<tr>
<td>Usoro</td>
<td>85</td>
</tr>
<tr>
<td>Von Roenn</td>
<td>63</td>
</tr>
<tr>
<td>Widmer</td>
<td>48</td>
</tr>
<tr>
<td>Wilkin</td>
<td>74</td>
</tr>
<tr>
<td>Woodberry</td>
<td>50</td>
</tr>
<tr>
<td>Wu</td>
<td>S4</td>
</tr>
<tr>
<td>Yuan</td>
<td>9</td>
</tr>
<tr>
<td>Zheng</td>
<td>67</td>
</tr>
</tbody>
</table>
SHORT INTERFERING RNAs AND RNA INTERFEERENCE

Phillip A. Sharp, Center for Cancer Research, Department of Biology and the McGovern Institute for Brain Research, Massachusetts Institute of Technology, Cambridge, MA 02139

RNA Interference (RNAi) has greatly expanded the concept that nucleic acids can direct gene-specific regulation and may in the future lead to new approaches to treat diseases such as AIDS caused by HIV. RNAi was initiated by the discovery of Andy Fire of the critical role of double strand RNA in the silencing of genes. The key intracellular intermediates between long double strand RNAs (dsRNAs) and specific silencing of a gene are short RNAs (siRNAs), which are processed from the longer substrate by an RNase III-type endonuclease called DICER. In general, siRNAs have proven to be effective in silencing many different genes in a variety of mammalian cell types. The search for siRNAs in different organisms led to the discovery of a large family of endogenous short single strand RNAs which are probably involved in gene regulation. The two prototypes of these short RNAs are lin-4 and let-7 of C. elegans which control developmental transitions in worms by inhibition of translation of specific mRNAs. These 21nt RNAs partially base pair to the 3’ untranslated region of their target mRNAs. There are over 200 genes encoding these short RNAs, microRNAs, in the mammalian genome. Expression of these genes as miRNAs can be cell type specific. Recent results suggest that siRNAs when transfected into mammalian cells are recognized by the same cellular pathway which mediates translational repression by the endogenous microRNAs. This suggests that siRNAs will be active in most if not all cell types and organs of humans. It further suggests that the partial pairing of siRNAs to mRNAs may generate gene inhibition that would complicate the use of these entities to silence a specific target gene. This potential off target complication is probably not a major limitation of the use of siRNAs because lentiviral vectors have been used to introduce genes which specify the synthesis of siRNAs and thus RNAi in most tissues of a mouse.
CLONAL SELECTION FOR TRANSCRIPTIONALLY ACTIVE VIRAL ONCOGENES DURING PROGRESSION TO CANCER

B.A. Van Tine1,2, J.C. Kappes3,4, N.S Banerjee2, J. Knops5+, L. Lai3, R.D.M. Steenbergen6, C.L.J.M. Meijer6, P.J.F. Snijders6, P. Chatis7,8, P.T. Moen, Jr.7*, L.T. Chow2 and T.R. Broker2. Departments of Pathology1, Biochemistry and Molecular Genetics2, Medicine3, Microbiology4, Laboratory of Medical Genetics5, University of Alabama at Birmingham, Birmingham, AL, 35294. 6Department of Pathology, Vrije Universitat Medical Center, Amsterdam, The Netherlands. 7New Technologies Research and Development Group, PerkinElmer Life Sciences, Inc., Boston, MA 02118, 8Division of Infectious Disease, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA.

Primary keratinocytes immortalized by human papillomaviruses (HPVs) and HPV-associated cervical carcinoma cell lines, such as CaSki and SiHa, are excellent models for investigating neoplastic progression to cancer. By simultaneously visualizing viral DNA and nascent viral transcripts in interphase nuclei following tyramide fluorescence in situ hybridization (T-FISH), we demonstrated for the first time, a selection for a single papillomavirus transcription domain (PVTD) independent of the number of integrated viral DNA copies. The expression invariably comes from the 3′ boundary of the virus-host fusion and involves a viral copy deleted of at least a portion of the E2 gene. The PVTD did not associate with several known subnuclear addresses but was almost always peri-nucleolar. Some silent copies were activated by growth in 5-azacytidine which inhibits DNA methylation. HPV-immortalized cells further transduced with HPV oncogenes and selected for marker gene co-expression transcribed only the newly introduced genes. Over-expression of E6-E7 oncogenes appears to drive the cells into crisis. Thus, transcriptional selection in response to environmental changes is a dynamic process for achieving optimal gene expression for cell survival. 13/13 cases of HPV-associated cancers also exhibited a single transcription center. This phenomenon may be critical in clonal selection during carcinogenesis. The silencing process offers a rational explanation for resolving the breakage-fusion bridge cycle described in the Abstract from Louise Chow on mechanisms for HPV DNA segregation.
HPV IMMUNITY AND VACCINE DEVELOPMENT

Douglas Lowy, Petra Lenz, Cynthia Thompson, Susana Pang, Patricia Day, Diana Pastrana, Christopher Buck, and John Schiller. Laboratory of Cellular Oncology, National Cancer Institute, Bethesda, MD 20892.

Papillomaviruses cause benign and malignant diseases of the skin and mucous membranes, including cervical cancer, which is the second most common cancer of women worldwide. Systemic immunization with candidate prophylactic vaccines composed of papillomavirus virus-like particles (VLPs) in preclinical models can induce high titers of type-specific neutralizing antibodies even without adjuvant and confer protection against high dose challenge with the homologous papillomavirus. Recent human vaccine trials of VLPs from HPV types implicated in human cancer have found that systemic immunization is well tolerated and highly immunogenic, inducing a predominantly Th1 response without adjuvant, as determined by IgG subtype. Proof of principle efficacy trials have also shown excellent protection against persistent genital HPV infection, which is a closely associated risk factor for premalignant cervical lesions and cervical cancer. To study the basis for the immunogenicity of papillomavirus VLPs, their interaction with antigen presenting cells (APCs) has been explored. VLPs were efficiently bound and internalized by mouse bone marrow derived dendritic cells, leading to phenotypic and functional activation of these APCs. The release of pro-inflammatory cytokines was slow, which may explain the relative lack of local reactogenicity seen in the clinical trials. VLPs also induced activation of human peripheral blood mononuclear cells, with the most robust release of cytokines being induced in the more differentiated APCs, macrophages and dendritic cells. These results are consistent with the pronounced Th1 response seen in people after VLP vaccination.
DEVELOPMENT OF HPV DNA VACCINES FOR AIDS PATIENTS

T.-C. Wu, The Johns Hopkins Medical Institutions, Baltimore, Maryland 21205.

The prevalence of human papillomavirus (HPV)-related lesions is significantly increased in AIDS patients with decreased CD4+ T cell counts. Thus, an effective therapeutic HPV vaccine strategy for AIDS patients should focus on enhancing HPV-specific CD8+ T cell immune responses in a CD4-independent manner. DNA vaccines have emerged as an attractive approach for antigen-specific immunotherapy and intradermal administration of DNA vaccines via gene gun represents an efficient way to deliver DNA vaccines into professional antigen-presenting cells \textit{in vivo}. We have successfully used the gene gun delivery system to test several DNA vaccines that employ intracellular targeting strategies to enhance MHC class I and class II presentation of encoded HPV-16 E7 antigen. The impressive pre-clinical data generated from our studies have led to several HPV DNA vaccine clinical trials tentatively scheduled to begin in late 2003. More recently, we have tested several anti-apoptotic factors for their ability to enhance dendritic cell survival and HPV-16 E7-specific CD8+ T-cell immune responses using DNA vaccination via gene gun. Administration of E7-expressing DNA with DNA encoding anti-apoptotic agents resulted in prolonged dendritic cell survival and an increased number of E7-expressing dendritic cells in the draining lymph nodes, which resulted in enhanced activation of E7-specific CD8+ T-cells in vaccinated mice. We have also further enhanced the potency of DNA vaccines by combining the anti-apoptotic strategies with intracellular targeting strategies. Such approaches resulted in a significantly enhanced CD8+ T-cell mediated immune response and anti-tumor effects against an E7-expressing murine tumor model. More importantly, this enhanced activation of E7-specific CD8+ T cells was also observed in CD4-knockout mice. Thus, our data based on these strategies have important clinical implications for the future therapeutic vaccination of AIDS patients against HPV infections and HPV-associated lesions.
S5
MOLECULARLY DEFINED CANCER VACCINES BASED ON INSIGHT IN T-CELL-DENDRITIC CELL INTERACTIONS
Cornelis JM Melief, Dept. Immunohematology and Blood Transfusion, Leiden University Medical Center, PO Box 9600, 2300 RC Leiden, The Netherlands. e-mail: cmelief@lumc.nl
* Emory University, Atlanta, USA

Molecular triggers of DC activation sufficient for induction of CD8+ CTL responses include agonistic CD40 antibody or ligands of Toll like receptors such as LPS (TLR4 ligand) or CpG (TLR9 ligand). In natural immune responses specific CD4 cells, reactive with peptide antigens presented by MHC class II molecules on DC, can also drive maturation of immature DC to the mature DC state required for CD8+ CTL response induction. CD4+ T helper cells to a large extent operate through upregulation of CD40L which then interacts with CD40 on DC to cause the required DC activation. Important cognate interactions for full CD8+ CTL induction by activated DC are CD80/CD86 on the DC, costimulating CD28 on the CD8 cells. For maintenance and full expansion of CD8+ T cells, interaction of 4-1 BBL (another member of the TNF(R) family) on DC with 4-1 BB on CD8+ CTL is also important. In the absence of CD80/CD86 costimulation, the 4-1 BBL -> 4-1 BB interaction appears to be inactive. Thus proper induction, expansion and maintenance of CD8+ CTL responses involves delicate interactions between CD4+ T-cells, DC and CD8+ T-cells involving several members of the TNF(R) family, including as signal transduction molecules CD40 on DC and 4-1 BB as well as CD27 on CD8+ CTL precursors. To prevent untoward destruction of antigen bearing DC by activated CD8+ CTL, DC protect themselves by upregulation during maturation of SPI-6, a member of the serpin family that specifically inactivates granzyme B and thereby blocks CTL-induced apoptosis. Interestingly, T helper 1 cells, which best induce CTL responses, cause SPI-6 expression and subsequent DC resistance. In contrast T helper 2 cells neither induce SPI-6 nor resistance to CTL mediated lysis of DC.

We recently investigated the conditions for optimal therapeutic CD8+ CTL induction by long peptide vaccins against human papillomavirus induced mouse tumors. The 32-35 amino acid long peptides were given SC in IFA or in CpG 1826 adjuvant. Powerful therapeutic CTL induction by single peptide vaccination crucially depends on coinjection at the same site of CpG adjuvant and this response was MHC class II independent. In prime-boost regimes a second mechanism started contributing to CTL induction, namely CD4+ T helper cell mediated CD40L dependent activation of DC. Toll like receptor triggering is therefore very useful in CD8+ CTL priming, while CD40L activation starts operating in boosting.

In addition, quite apart from their activation of CD4+ helper cells, long peptides are superior to exact MHC class I binding peptides. It appears that the long peptides generate intracellular reservoirs of antigen for MHC class I & II processing, ensuring consistent and prolonged cell surface display of MHC-bound peptides. Exact MHC class I binding peptides, in contrast, show only a short half life at the cell surface. The combined data show that a new powerful generation of therapeutic anti-cancer vaccines consists of completely synthetic compounds: specific synthetic peptides and synthetic CpG adjuvants.
S6
Abstract not submitted.
HPV TYPE-SPECIFIC AND PHYLOGENETIC DIFFERENCES IN THE EFFECTS OF IMMUNE STATUS
Howard D. Strickler, MD, MPH, Department of Epidemiology & Social Medicine, Albert Einstein College of Medicine, Bronx, NY

Recent studies of HPV natural history in HIV-positive women indicate that the effects of immune status on prevalence and incidence of HPV infection vary by HPV type. These results have important clinical and biologic implications.

Utilizing specimens and data obtained from two large prospective cohort studies, the Women’s Interagency HIV Study (WIHS) and the HIV Epidemiology Research Study (HERS), we have observed that HPV 16, the HPV type associated with approximately half of cervical cancers, has a weak association with CD4+ T-cell strata compared with most other HPVs. This is consistent with the hypothesis that: (i) HPV 16 may avoid immune surveillance better than other HPV types and, therefore, (ii) HPV 16 infection may be more independent of the effects of immune status. HPV 33 and 35, two HPV 16-related types, also had relatively weak associations with immune status, and preliminary results suggest that additional phylogenetic patterns may exist: three out of the four A5 genus HPV types (i.e., HPV 69, 82, and 26) were, respectively, the first, third and fourth most strongly associated with CD4+ T-cell levels out of a total of 32 HPV types detected.

Preliminary analyses further indicate that recent (but not lifetime) sexual behavior is significantly associated with “incident” detection of HPV in HIV+ women, suggesting that a substantial portion of the high rate of new HPV in HIV+ women may be due to recent acquisition rather than reactivation. In multivariate analyses, controlling for HIV and CD4+ strata, incident infection was also significantly associated with bacterial vaginosis, trichomoniasis, and smoking. The findings were not altered by different approaches to controlling for treatment of cervical neoplasia, including censoring, however, treatment itself was found to increase risk of subsequent new HPV infection. Thus, the long follow-up and high rates of HPV in studies such as the WIHS and HERS is proving helpful in identifying cofactors that may affect HPV natural history, beyond the effects of HIV and immune status.

From a clinical perspective, the weak effects of immune status on HPV 16 infection may help explain why cervical cancer rates are only moderately increased in HIV+ women. An important corollary could also be true: improved immune status (e.g., through the use of HAART) would be expected to have only a moderate effect on risks of severe cervical neoplasms and cancer. In keeping with this, we predict that HAART usage will reduce the persistence and progression of cervical lesions, but that its impact will be less on lesions related to HPV 16 compared with other HPV types. It should be possible in the near future to collect appropriate data to test these predictions. Analysis of the DNA sequences of HPV types strongly versus weakly associated with immune status may reveal biologic correlates that could explain such differences; data that might prove useful in identifying epitopes relevant to the immunologic control of HPV infection.
KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS (KSHV) MOLECULAR EPIDEMIOLOGY OF MALIGNANCY IN ORGAN TRANSPLANTATION

Mario Luppi, Patrizia Barozzi, Fabio Facchetti, Ronit Sarid, Valeria Rasini, Luisa Ravazzini, Carlotta Spano, Daniela Vallerini, Giovanni Riva, Raffaella Bosco, Giulia Fontana, Dana G Wolf, Thomas F. Schulz, Giuseppe Torelli.

Department of Oncology and Hematology. Section of Hematology. University of Modena and Reggio Emilia. Modena, and 1Department of Pathology, University of Brescia, Brescia, Italy. 2Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan, and 3Department of Clinical Microbiology and Infectious Diseases, Hadassah University Hospital, Jerusalem, Israel. 4Department of Virology, Hannover Medical School, Hannover, Germany.

We tested the hypothesis that the post-transplant Kaposi’s sarcoma (KS), which rather frequently develops in renal transplant recipients, may be of donor origin, similarly to the post-transplant lymphoproliferative diseases (PTLD) occurring in recipients of bone marrow grafts, which are known to result from the expansion of Epstein-Barr virus (EBV) infected B cells of donor origin. We used either single cell polymerase chain reaction (PCR) or microsatellite analysis to examine the cutaneous KS lesions which have developed 9-40 months after renal transplantation in six female recipients of kidneys from male Italian donors and in two male recipients of kidneys from male donors of Arab and Jewish origin respectively. The spindle and endothelial cells were identified as cells positive for the CD34 antigen and for the latent human herpesvirus-8 (HHV-8) antigen LNA1/orf73. Single stained cells were isolated by micromanipulation and subjected to PCR analysis for the Y chromosome and/or HLA genes of the male donors as well as for a specific genomic sequence (orf-26) of HHV-8. We detected the presence of genetic markers of the donors (chromosome Y and/or HLA gene) in the spindle and endothelial HHV-8 infected cells localized in the cutaneous KS lesions, indicating, unequivocally, that the KS neoplastic cells were of donor origin. The PCR data were confirmed by microsatellite analysis, showing the presence of the polymorphic profile of donor in the microdissected KS tissue, while the polymorphic profile of the recipient was identified in the unaffected epidermis and/or in the peripheral blood. The combination of both molecular techniques, allowed us to demonstrate the donor origin of the bulk of the KS tumor in five out of eight transplant patients. In one of these five cases, from which frozen tissue was available, immunohistochemical and double immunofluorescence experiments also showed the expression of donor HLA antigen in the large proportion of neoplastic, LNA1/orf73 expressing cells, being the recipient HLA antigen expression confined only to the unaffected epidermis or to the non neoplastic subdermal vessels. Moreover, in three out of these five cases, it was possible to prove that also the viral sequences in the neoplastic lesions were of donor origin.

Our study shows, for the first time, that not only the KS associated herpesvirus but also the still elusive, possibly hemopoietic, KS progenitor cell may be seeded with a solid organ transplantation, survive in a recipient host and transform. It is well known that KS is under immune control and patients who have undergone transplantation often have regression of KS with decrease in their immunosuppressive regimens. The findings reported here indicate HHV-8 positive post-transplant KS as the second known example of a virus associated tumor transmitted through transplantation, providing the rationale for the therapeutic use of donor HHV-8 specific cytotoxic T cells.
S9
EPIDERMODYSPLASIA VERRUCIFORMIS: FIRST IDENTIFICATION OF HUMAN GENES CONDITIONING THE SUSCEPTIBILITY TO CUTANEOUS ONCOGENIC PAPILLOMAVIRUSES
Nicolas Ramoz¹, Luis-Alfredo Rueda², Bakar Bouadjar³, Stefania Jablonska⁴, Michel Favre¹, Gérard Orth¹. ¹Institut Pasteur, Paris, France, ²Bogota, Colombia, ³Algiers, Algeria and ⁴Warsaw, Poland.

Epidermodysplasia verruciformis (EV) is a rare autosomal recessive genodermatosis associated with a high risk of skin carcinoma that results from an abnormal susceptibility to infection by specific human papillomaviruses (HPVs). EV HPVs are considered to be innocuous for the general population but induce persistent wart-like or macular lesions in EV patients. Skin carcinomas, usually associated with the oncogenic HPV5, develop in about half of the cases. EV thus represents a model disease to analyze the genetic basis for interindividual variation in susceptibility to HPV infections. A genetic linkage analysis performed on consanguineous EV families allowed us to map a susceptibility locus for EV to chromosome 17q25. We have now identified nonsense mutations segregating with EV in either of two adjacent putative novel genes (EVER1 and EVER2) contained in this region. This represents the first identification of genes conditioning susceptibility to HPV infections. cDNAs of full-length EVER1 and EVER2 transcripts were characterized from lymphoblastoid cell lines and from normal skin. Putative EVER1 and EVER2 proteins share 28.4% amino acids and show no homology with known human proteins. Both of them have features of integral membrane proteins and are localized in the endoplasmic reticulum. It remains to be determined whether EVER1 and EVER2 control the interaction between epidermal keratinocytes and HPVs specifically associated with EV or are involved in a specific way in the innate or adaptive immune responses leading to the eradication of infected keratinocytes.
S10

EPSTEIN BARR VIRUS AND CANCER
Nancy Raab-Traub. Lineberger Comprehensive Cancer Center, The University of North Carolina, Chapel Hill, NC 27599-7295

The Epstein-Barr virus (EBV) contributes to the development of several human cancers, including post-transplant lymphoma, a subset of AIDS related lymphomas, Hodgkin's lymphoma, and nasopharyngeal carcinoma (NPC). These malignancies are clonal proliferations that develop from an EBV-infected cell. Viral expression differs in the various pathologies associated with EBV, however, in most of the malignancies, the viral genes expressed include the EBV latent membrane proteins 1 and 2 (LMP1 & 2), the EBV nuclear antigen 1, the EBER transcripts, and a family of RNAs encoded by the BamHI A EBV fragment.

LMP1 has transforming properties in rodent fibroblasts and is essential for EBV induced transformation of lymphocytes. It is expressed in NPC and in rare, early neoplastic nasopharyngeal lesions. LMP1 interacts with the signaling molecules for the tumor necrosis factor family of receptors (TRAFs) and is considered a constitutively activated member of this receptor family. This interaction activates the NFkB transcription factor and results in profound effects on cellular gene expression. Transgenic mice that express LMP1 under the control of an immunoglobulin heavy chain promoter and enhancer develop lymphoma with 3-4 times increased incidence than that of age-matched negative controls. These data indicate that LMP1, without expression of other EBV genes, is oncogenic in vivo and suggest that LMP1 is a major contributing factor to the development of EBV-associated lymphomas. To evaluate genetic factors that may contribute to oncogenesis, the LMP1 transgenic mice have been crossed with genetically engineered mice to evaluate the potential loss of the p16 tumor suppressor gene. The effects on tumorigenesis will be presented.

A second gene expressed in EBV associated tumors is LMP2 which is not essential for B cell transformation. In epithelial cell lines grown in vitro, LMP2 induces proliferation and inhibits differentiation with activation of the serine-threonine kinase, Akt. In vivo, activated Akt was detected in rare Reed-Steinberg cells in EBV negative lymphomas but was readily detected in most of the Reed-Steinberg cells in EBV positive samples and in the C15 NPC tumor. These data suggest that LMP2 is expressed in HD and NPC and that one consequence of this expression is activation of the Akt kinase.

One target of the Akt kinase is glycogen synthase kinase 3 beta (GSK3B) which phosphorylates and induces degradation of beta catenin. Expression of LMP2 and activation of Akt in epithelial cells leads to the accumulation and translocation of Akt to the nucleus. In vivo, activated Akt and nuclear beta catenin has been detected in the C15 NPC tumor. These data suggest that LMP2 is expressed in NPC with activation of Akt and beta-catenin.

The consistent expression of multiple viral proteins in EBV-associated malignancies and the development of animal models and tissue culture models that retain characteristics of these tumors provide new opportunities for the development and testing of anti-viral therapies targeted to specific viral:cell protein interactions and virally-activated signaling pathways.
A NOVEL VIRAL MECHANISM FOR DYSREGULATION OF β-CATENIN IN KAPOSI’S SARCOMA HERPESVIRUS ASSOCIATED LATENCY

Masahiro Fujimuro, Frederick Y. Wu, Colette apRhys, Henry Kajumbula, Gary S. Hayward and S. Diane Hayward. Sidney Kimmel Cancer Center, Johns Hopkins School of Medicine, Baltimore, MD 21231.

Kaposi’s sarcoma-associated herpesvirus (KSHV) is associated with Kaposi’s sarcoma, primary effusion lymphoma and multicentric Castleman’s disease in AIDS patients. The latency associated nuclear antigen LANA is expressed in each of these malignancies. LANA is essential for replication and maintenance of KSHV episomes during latent infection. LANA is also believed to contribute to KSHV-associated tumorigenesis although the mechanisms involved are not fully understood. We present evidence that LANA stabilizes beta-catenin, the nuclear effector of the Wnt signaling pathway, and stimulates S-phase entry. Abundant beta-catenin was detected in primary effusion lymphoma cell lines and in Kaposi’s sarcoma tissue samples by western blot and immunohistochostchemical analyses. Introduction of anti-LANA siRNA into PEL cells eliminated beta-catenin accumulation indicating that LANA was responsible for the beta-catenin dysregulation. LANA was found to stabilize beta-catenin by a novel mechanism involving glycogen synthase kinase-3beta. In canonical Wnt signaling, glycogen synthase kinase-3beta, phosphorylates beta-catenin and targets it for proteasomal degradation. A yeast two-hybrid assay identified glycogen synthase kinase-3 as a LANA binding protein. LANA redistributes glycogen synthase kinase-3beta such that the enzyme becomes disproportionately nuclear. A mutant LANA that was unable to bind to and redistribute glycogen synthase kinase-3beta was also unable to stabilize beta-catenin. We further demonstrate that LANA stimulates cells to enter S-phase and that this property is dependent upon the ability to bind to glycogen-synthase kinase-3beta. This study identifies stabilization of beta-catenin as a key mechanism by which LANA contributes to KSHV-associated tumorigenesis.
THE PAPILLOMAVIRUS E6 PROTEIN AND C-MYC CO-MODULATE THE hTERT PROMOTER AND INDUCE CELLULAR TELOMERASE

Xuefeng Liu, Hang Yuan, Tim Veldman and Richard Schlegel. Georgetown University Medical School, Washington, DC, 20057.

The papillomavirus E6 protein binds and directs the ubiquitin-dependent degradation of the p53 tumor suppressor protein. Independent of this degradative function, however, E6 induces cellular telomerase activity. This increase in enzyme activity reflects E6-enhanced transcription of the telomerase hTERT catalytic subunit, but the molecular basis for this transactivation is unknown. We demonstrate that E6/Myc interactions regulate hTERT gene expression. Mad protein, a specific antagonist of Myc, repressed E6-mediated transactivation of the hTERT promoter and this repression was relieved by Myc overexpression. The proximal Myc/Max binding element (E-box) in the hTERT promoter was the major determinant of both E6- and Myc-responsiveness in keratinocytes. E6 did not alter Myc protein expression or Myc/Max association, and the induction of hTERT by Myc/E6 was independent of Myc phosphorylation at Thr58/Ser62 within the transactivation domain. However, immunoprecipitation studies demonstrated that endogenous Myc protein co-precipitated with E6 protein. More importantly, chromatin immunoprecipitation analyses demonstrated that both E6 and Myc proteins bound to a minimal 295 bp hTERT promoter in vivo. The observation that E6 physically associates with Myc complexes and activates a Myc-responsive gene identifies a new mechanism by which this oncogene can modulate cell proliferation and differentiation.
MECHANISM OF GENETIC LESION IN AIDS-ASSOCIATED LYMPHOMA

Riccardo Dalla-Favera, Institute for Cancer Genetics, Columbia University

Substantial evidence suggest that the pathogenesis of AIDS-related non-Hodgkin lymphomas (AIDS-NHL) is associated with chromosomal translocations that deregulate the expression of various oncogenes. Recently, a novel mechanism of genetic lesion, termed aberrant hypermutation, has been identified in diffuse large B-cell lymphoma (DLBCL) of immunocompetent hosts. In these tumors, the somatic hypermutation process (SHM) that normally targets immunoglobulin V genes (IgV) in B-cells appears to misfire and causes mutations in the 5' sequences of multiple proto-oncogenes, including PIM-1, PAX-5, RhoH/TTF and c-MYC. We have recently found that the aberrant hypermutation is also associated with AIDS-NHL where its activity found in most major histologic subtypes. The number of oncogenes found mutated and the percentage of cases involved suggest that this mechanism may represent a major contributor to AIDS-NHL pathogenesis. To explore further the pathogenesis of these malignancies, we have used gene expression profiling to address the following questions: i) does the morphological classification of AIDS-NHL reflect all the biological entities of these tumors? ii) what are the normal cellular counterparts of the various AIDS-NHL subtypes? iii) are AIDS-NHL tumor cells different from their counterparts occurring in immunocompetent hosts?
Diffuse large B-cell lymphoma (DLBCL), the most common lymphoid malignancy in adults, is currently curable in only 40% of patients. Clinical prognostic factor models such as the International Prognostic Index identify patients who are unlikely to be cured with standard therapy. However, these clinical models do not provide additional insights regarding more effective treatment strategies. In the absence of molecular insights into the observed heterogeneity of DLBCL, therapeutic approaches to “high-risk” patients have largely focused on modifying doses and schedules of conventional chemotherapeutic agents and adding stem cell support. However, these approaches have not significantly improved DLBCL patient survival, underscoring the need to identify more rational, molecularly defined approaches to treatment. The clinical features used to identify “high-risk” DLBCL are likely to surrogates for intrinsic molecular heterogeneity in the disease. The recent development of DNA microarrays provides an opportunity to take a genome-wide approach to identifying molecular signatures of previously unrecognized DLBCL subsets and prognostic categories. Recent studies indicate that supervised learning classification techniques can be used to predict outcome in DLBCL and identify rational targets for intervention. The rapidly evolving area of gene expression profiling in DLBCL will be reviewed with particular emphasis on newly identified disease subsets and novel treatment targets.
S15

SPLENIC LYMPHOMA WITH VILLOUS LYMPHOCYTES IN PATIENTS WITH CHRONIC HEPATITIS C VIRUS INFECTION
Olivier Hermine, François Lefrère, Xavier Troussard.
Hôpital Necker Paris and CHU Côte de Nacre Caen, France.

Introduction: Epidemiological studies have suggested a link between hepatotrophic hepatitis C virus (HCV) infection and B-cell non-Hodgkin lymphoma (NHL), particularly in Immunocytoma/lymphoma and in primary hepatosplenic large B-cell lymphoma. The role of the virus in lymphomagenesis has never been demonstrated. Therefore, we have conducted a study to define the role of HCV load in the splenic lymphoma with villous lymphocytes subgroup of B-cell NHL pathogenesis by treating HCV positive patients with antiviral therapy.

Patients and Methods: Patients with splenic lymphoma with villous lymphocytes and positive for HCV (SLVL/HCV+) infection were treated with antiviral therapy by alpha-interferon (3 Millions International Units (MIU) 3xwk) or alpha-interferon (3MIU 3x/wk) and Ribavirin (1000mg/d), and the outcomes were compared with those of six similarly treated splenic lymphoma with villous lymphocytes /HCV negative (SLVL/HCV-) patients identified retrospectively by record review.

Results: Nine patients with SLVL/HCV+ were treated by interferon. Seven patients entered into complete remission after achieving a negative HCV PCR. The two refractory patients achieved partial and complete remission after the addition of ribavirin and achievement of a negative HCV PCR. A relapse occurred in one patient when HCV load was once again positive by PCR. In contrast, none of the six SLVL/HCV- patients responded to interferon therapy.

Discussion: This data strongly suggests that treatment with interferon is beneficial for patients with HCV infection and concurrent SLVL, and that HCV infection plays a direct role in SLVL/HCV+ lymphomagenesis. Recent epidemiological studies seem also to demonstrate a link between HCV and SLVL. These findings may be relevant to patients with other types of lymphoma who are concomitantly infected by HCV. The mechanisms by which HCV leads to the development of B-cell lymphoma remain to be determined. It may exert its oncogenic potential via an indirect mechanism by chronic antigenic stimulation. However, a direct oncogenic role of HCV proteins is not excluded. Therefore, we believe that screening for HCV should be considered at diagnosis for patients with low grade B-cell lymphoma and, although clinical implications remain to be defined in those with high grade B-cell lymphoma. Because of its clinical implication, if our data are confirmed in a larger group of patients, HCV related low grade lymphoma should be classified as a special entity as it has been proposed in the World Health Organization for T-cell lymphoproliferation related to human T-cell lymphotrophic virus type 1.
DOSE-ADJUSTED EPOCH CHEMOTHERAPY (DA-EPOCH) IN AIDS-RELATED NON-HODGKIN’S LYMPHOMA (ARL): THE NATIONAL CANCER INSTITUTE EXPERIENCE

Richard F. Little, Robert Yarchoan, and Wyndham H. Wilson, NCI

In a study of DA-EPOCH with HAART suspension in 39 patients with untreated ARL, 74% achieved CR and at 53 months median follow-up, disease-free and overall survival are 92% and 60%, respectively. Examination of tumor histogenesis and biological markers of tumor resistance may help explain this outcome. ARL lymphogenesis, like other immune-related lymphoproliferative diseases, is associated with the underlying immune pathology; specifically low CD4 ARL are associated with immunoblastic (IBL) diffuse large B-cell lymphomas (DLBCL) and gammaherpesviruses, and poor clinical outcome, whereas higher CD4 ARL are associated with centroblastic DLBCL and Burkitt’s, and a better outcome. Biologically, IBL DLBCL appear to primarily derive from a post-germininal center (PGC) origin, and centroblastic DLBCL and Burkitt’s are primarily of germinal center (GC) origin. Recent microarray studies indicate PGC DLBCL is more drug resistant, possibly due to constitutive NFêB and BCL-2 expression. We examined markers of drug resistance in our cases, including MIB-1 (proliferation), BCL-2 and p53 (anti-apoptosis), and found differences in HIV+ and HIV– DLBCL. HIV+ DLBCL cases had higher MIB-1, a feature associated with poor outcome with CHOP but not with EPOCH (Wilson et al. Blood 1997; 89: 601-9), whereas HIV+ cases had lower BCL-2. Interestingly, unlike HIV– DLBCL, p53 overexpression was unrelated to drug resistance in HIV+ cases. We hypothesize that the improvement in ARL with HAART is likely due to an upward CD4 risk migration, which favors lymphoma GC histogenesis and lower drug resistance. EPOCH efficacy may be partially explained by its efficacy in DLBCL with high MIB-1. These results emphasize the importance of tumor biology in treatment outcomes and suggest that by forestalling immune depletion, HAART has shifted tumor pathogenesis and confers no specific benefit during chemotherapy treatment.
NO BENEFIT FROM RITUXIMAB IN A RANDOMIZED PHASE III TRIAL OF CHOP WITH OR WITHOUT RITUXIMAB FOR PATIENTS WITH HIV-ASSOCIATED NON-HODGKIN'S LYMPHOMA: AIDS MALIGNANCIES CONSORTIUM STUDY 010

LD Kaplan for the AIDS Associated Malignancies Consortium.

To determine whether the addition of rituximab to CHOP results in a higher response rate, individuals with HIV-associated aggressive B-cell lymphoma were randomized in a 2:1 fashion to receive standard-dose CHOP with rituximab (group A, n=95) or CHOP alone (group B, n=47). All patients received cyclophosphamide 750mg/m², doxorubicin 50mg/m², vincristine 1.2mg/m² and prednisone 100mg qd x 5 days. Those in arm A received rituximab d1 and CHOP d3 of each cycle. Three monthly maintenance doses of rituximab were administered in group A following completion of chemotherapy. All patients received G-CSF support beginning in cycle 1. Patients were restaged every 2 cycles.

Mean age was 42 (26-73). Median CD4+ lymphocyte count was 133/mm³. Serum LDH was elevated in 97/142 (68%) and 109/138 (79%) had stage III or IV disease. There were no significant differences in these baseline characteristics between the two study arms.

Complete response (CR+CRu) was achieved in 58% and 50% of patients in arms A and B respectively (p=0.371) with median times to response of 8.5 and 9 weeks respectively. With a median of 26 weeks of follow-up, median response duration has not been reached in either group. Grade 3/4 neutropenia was reported in 37/95 (39%) of group A and 8/47 (17%) of group B patients (p=0.012). Death due to infection occurred in 14/95 (15%) group A (including sepsis in 6) and only 1/47 (2%) group B patients (p=.02). Overall, fever with ANC< 1000/mm³ occurred in 24/95 (25%) patients in group A and 4/47 (8.5%) group B patients (p=0.024). Documented infection in the setting of neutropenia occurred in 10 and 0 patients in groups A and B respectively (p=0.031).

Preliminary results of this trial indicate no benefit to the addition of rituximab to CHOP for the initial treatment of individuals with HIV-associated non-Hodgkin’s lymphoma while the high incidence of neutropenic infection and death in those receiving rituximab raise serious concerns regarding the safety of this approach in this patient population.
CANCER RISK IN THE WOMEN’S INTERAGENCY HIV STUDY (WIHS). NA Hessol 1, EC Seaberg 2, S Preston-Martin 3, LS Massad 4, HS Sacks 5, S Silver 6, S Melnick 7, AM Levine 3.

1UCSF, San Francisco, CA; 2Johns Hopkins, Baltimore, MD; 3USC, LA, CA; 4Rush Medical College, Chicago, IL; 5Mt Sinai School of Medicine, NY, NY; 6George Washington University, Washington, DC; 7NCI, NIH, Bethesda, MD.

Background: The HIV epidemic has been associated with an increased incidence of specific types of cancers. However, less is known about cancers occurring in HIV-infected women than men.

Methods: To determine the risk of cancer among HIV-infected and at-risk HIV-uninfected women, we compared cancer incidence data from the WIHS to data from the US Surveillance, Epidemiology, and End Results (SEER) registry. Age and race–adjusted standardized incidence ratios (SIR) were computed. To evaluate the effect of highly active antiretroviral therapy (HAART), cancer incidence during the pre-HAART and HAART eras were compared.

Results: Among the 1950 women participants (1554 HIV-infected, 391 HIV-uninfected, and five HIV seroconverters), 48 cancers were diagnosed during study follow-up. Among HIV-infected women, significantly increased incidence rates were observed for KS (SIR=218.6), NHL (SIR=23.2), and lung cancer (SIR=9.7) when compared to SEER rates. Lung cancer incidence was also significantly elevated among the HIV-uninfected women (SIR=11.0), when compared to SEER rates, and was similar to the SIR for HIV-infected women. In multivariate regression analyses, the cancer incidence rate among HIV-infected women who initiated HAART was cut by 60% (rate ratio = 0.4, 95% confidence interval 0.2-0.9).

Conclusion: HIV-infected women had increased incidence rates for KS and NHL, but not for invasive cervical cancer. Both HIV-infected and uninfected women had increased incidence rates of lung cancer, when compared to population-based expected rates. The adjusted overall cancer incidence rate was significantly lower in women following HAART initiation.
BREAST CANCER AMONG HIV-INFECTED WOMEN: FINDINGS FROM THE WOMEN'S INTERAGENCY HIV STUDY (WIHS)


1 University of Southern California, 2 Johns Hopkins School of Public Health, 3 The George Washington University Medical Center, 4 National Cancer Institute, 5 University of California, San Francisco, 6 Mount Sinai Medical Center

Background. A number of studies, including the WIHS, have found HIV-infected women to have lower than expected rates of breast cancer. We undertook to explain this apparent deficit by examining the distribution of various established breast cancer risk factors among women in our WIHS cohort. In addition, we reviewed the pathology slides of all WIHS women with prevalent or incident breast cancers and compared findings to those usual among US breast cancer patients of similar ages.

Methods. The WIHS is the largest cohort to date of HIV seropositive women (N = 2,058) with a comparison cohort of seronegative women (N = 568). These cohorts were enrolled between October 1994 and November 1995 through six clinical consortia throughout the United States. At baseline, women were asked about prevalent cancers; at each six monthly follow-up interview and WIHS clinic visit, women were asked about breast or other cancers which had been diagnosed since their last visit. Medical records and tumor registry files were used to verify these self-reported cancers. The number of incident breast cancers observed was compared to the number expected using a comparison with breast cancer rates reported by the SEER program among women of similar age and ethnicity. Distributions were examined of characteristics known to relate to breast cancer risk, including age at first birth, parity, alcohol intake, and indicators of social class, such as household income. Pathology slides from WIHS breast cancer patients were reviewed by a pathologist (MS) with expertise in diseases of the breast.

Results. Seven WIHS women reported prevalent breast cancers, and another seven developed confirmed incident breast cancers. The Standardized Incidence Ratio (SIR) for incident breast cancer among HIV+ WIHS women was 0.7. Fewer WIHS women, compared to NHANES women, were found to be at high risk of breast cancer based on a number of factors known to reduce risk: lower social class (83.5% of WIHS women had household incomes of $24,000 or less), early age at first childbirth (63.6% were 20 or younger), high parity (60.4% of WIHS women had 2 or more live births), and low alcohol intake (73.5% of WIHS women drank no or only a little alcohol). Comparisons will be made of these distributions with those seen among other groups of US women such as those in the National Health and Nutrition Examination Survey III (NHANES III), a random sample of US women. Slide review confirmed diagnoses on the original surgical pathology reports and showed that the spectrum of diagnoses was not unusual compared to US breast cancer patients. The most common diagnosis was ‘infiltrating ductal carcinoma’.

Conclusions. The apparent deficit of breast cancers among women in the WIHS can be explained by their overall lower risk, given the distributions in this population of a number of established breast cancer risk factors.
INVERSE ASSOCIATION BETWEEN HUMAN HERPESVIRUS 8 (HHV-8) ANTIBODY TITERS AND PRESENCE OF HHV-8 DNA. Laney AS1,3, Dollard SC3, Jaffe HW3, Offermann MK1, Spira TJ3, Gunthel CJ1, Pellett PE2,3, Cannon MJ3. 1Emory University, 2Cleveland Clinic Foundation, and the 3Centers for Disease Control and Prevention.

HHV-8 infection is necessary for the development of Kaposi's sarcoma (KS). To better understand the relationship between HHV-8 activity and the host immune response, we studied 90 men (45 with KS) seropositive for both HIV-1 and HHV-8. For each man, we analyzed data from 4 visits at 3-week intervals. During the 2-month follow-up, HHV-8 antibody titers, measured with 2 peptide-based enzyme-linked immunosorbent assays (ORF 65 and K8.1), were relatively constant in individuals, whereas HHV-8 DNA was present sporadically. Geometric mean antibody titers were higher in men with KS than in men without KS for both ORF65 (2877 vs. 690, p<0.001) and K8.1 (1452 vs. 740, p=0.03). HHV-8 DNA prevalence was higher in men with KS than in men without KS in both peripheral blood mononuclear cells (PBMC) (35% vs. 11%, p<0.001) and oral fluid (29% vs. 22%, p=0.13). In multivariate analyses that controlled for KS status and accounted for within-patient correlation, very high ORF65 antibody titers ($1:26,000) were inversely associated with the presence of HHV-8 DNA in PBMC (odds ratio (OR)=0.19; 95% confidence interval (CI), 0.07-0.53) and oral fluid (OR=0.18; 95% CI, 0.05-0.60), and very high K8.1 antibody titers ($1:26,000) were also inversely associated with the presence of HHV-8 DNA in PBMC (OR=0.50; 95% CI, 0.16-1.58) and oral fluid (OR=0.45; 95% CI, 0.13-1.49).

Thus, although HHV-8 antibody titers and DNA were associated at the group level (i.e., geometric mean titers and DNA prevalence were both higher in the KS group), they were inversely associated at the individual level. This suggests that HHV-8 antibody (or other immune function for which antibody is a marker) may limit HHV-8 replication. However, the inverse relationship between HHV-8 DNA and antibody titers diminished as the cut-off defining high titers was lowered, suggesting that the anti-HHV-8 immune response is only effective in clearing circulating virus when antibody titers reach very high levels.
USE OF SALIVA AS A LUBRICANT IN SEXUAL PRACTICES AMONG HOMOSEXUAL MEN: CLUES TO THE ROUTE OF KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS (KSHV) TRANSMISSION
Lisa M. Butler, Dennis H. Osmond, and Jeffrey N. Martin. University of California, San Francisco.

Background: The exact route of KSHV transmission among homosexual men is unclear. That KSHV is most commonly found in saliva lends biological plausibility that saliva is the principal conduit for transmission. Although much attention has been placed on kissing as a means of saliva exchange among homosexual men, it is unknown to what extent saliva is involved in other sexual acts.

Methods: Participants from the San Francisco Young Men’s Health Study, a population-based cohort study of homosexual men, were interviewed with regard to use of saliva as a lubricant in various insertive and receptive sexual practices.

Results: 258 men were examined; median age was 35, 75% were white, and 66% had ≥ 4 years of college. Saliva use was common during lifetime and recent practice of insertive and receptive acts:

| Use of Saliva as a Lubricant in Insertive and Receptive Sexual Acts |
|---------------------------------|-----------------|-----------------|
| Sexual act                      | Lifetime        | Prior 6 months  |
| (*IQR = 25th to 75th percentile) | (*IQR*)         | (*IQR*)         |
| Receptive anal intercourse      | 71%             | 31%             |
| Insertive anal intercourse      | 65%             | 31%             |
| Receptive fingering/fisting     | 81%             | 44%             |
| Insertive fingering/fisting     | 75%             | 46%             |

Even among men who followed safe sex guidelines by always using condoms, 44% used saliva for anal intercourse or fingering.

Conclusion: Homosexual men commonly use saliva as a lubricant in penile-anal intercourse and fingering/fisting. That this is not universal, however, might explain the non-uniformity of KSHV in homosexual men. Use of saliva in these acts must be considered a potential mode for KSHV transmission as well as other pathogens. Consideration is warranted for altering safe sex guidelines.
EXPOSURE TO SALIVA AND THE PREVALENCE OF HUMAN HERPESVIRUS 8 INFECTION AMONG MEN WHO HAVE SEX WITH MEN

Corey Casper\textsuperscript{1,2}, David Carrell\textsuperscript{1}, John S. Pauk\textsuperscript{1}, Franklyn D. Judson\textsuperscript{3}, Rhoda Ashley Morrow\textsuperscript{1}, Lawrence Corey\textsuperscript{1,2}, Anna Wald\textsuperscript{1}, Connie Celum\textsuperscript{1}. \textsuperscript{1}University of Washington, \textsuperscript{2}Fred Hutchinson Cancer Research Center, \textsuperscript{3}Denver Department of Public Health.

Introduction: Infection with Human Herpesvirus 8 (HHV-8) is common among men who have sex with men (MSM). The oropharynx may be important in HHV-8 transmission and acquisition, but previous studies have not extensively surveyed behaviors which result in exposure to saliva and their relationship to HHV-8 infection.

Methods: We assessed sexual practices in 526 MSM including behaviors resulting in possible exposure to oral secretions. Serum was tested for antibodies to HHV-8. 133 of 526 participants (25.3\%) were HHV-8 seropositive. In univariate analysis, greater than 100 lifetime sex partners, at least one HIV-positive partner, or more than 50 partners of unknown HIV status were each found to be associated with HHV-8 infection. The performance of oral sex on HIV-negative partners while using condoms was associated with a decreased odds of HHV-8 infection (OR 0.57, p=0.04). With HIV-positive partners or partners of unknown HIV status, the performance of oral sex with ejaculation and without a condom (OR 1.73, p<0.001), protected receptive anal sex (OR 1.43, p=0.02), unprotected insertive anal sex (OR 1.67, p<0.001), performance of rimming (OR 1.67, p<0.001), receipt of rimming (OR 1.50, p=0.03), and deep kissing (OR 1.60, p=0.03) were each associated with increased odds of HHV-8 seropositivity. In multivariate modeling, only deep kissing was significantly associated with HHV-8 seropositivity (2.3\% increase in odds with each additional partner of unknown HIV status with whom this behavior was practiced, p=0.04).

Conclusions: Our findings suggest that HHV-8 may be acquired though oral-oral contact.
BLOOD TRANSFUSION AND HUMAN HERPESVIRUS 8 INFECTION IN CHILDREN WITH SICKLE CELL DISEASE IN UGANDA

SM Mbulaiteye¹, RJ. Biggar¹, PM. Bakaki², R Pfeiffer¹, D Whitby¹, AM. Owor², E Katongole-Mbidde², J J. Goedert¹, C M. Ndugwa², E A. Engels¹

¹ National Cancer Institute, Bethesda, MD, USA
² Makerere University Medical School, Kampala, Uganda

Introduction: Human herpesvirus 8 (HHV-8) DNA can be detected in peripheral blood, but blood-borne transmission has not been demonstrated. We studied the risk of HHV-8 infection in highly transfused children with sickle cell disease in Uganda.

Methods: We enrolled 600 children (age 0-16 years) at Mulago Hospital, Kampala, from 11/2001 to 4/2002, about half (57%) were transfused (mean: 2.7 transfusions per child), by design. HHV-8 serostatus was determined using enzyme-linked immunoassays for antibodies against HHV-8 proteins K8.1 and orf73. We used logistic regression to test for association between HHV-8 serostatus and transfusion history and a Markov model to estimate the transmission risk per transfusion.

Results: HHV-8 antibodies were detected in 117 of 561 (21%) children based on K8.1 results. Seroprevalence among the never-transfused children rose from 6.4% in those aged 0-2 years to 32.1% in children aged 13-16 years (p trend <0.001). HHV-8 seroprevalence was higher in transfused than never-transfused children (24% vs. 17%, p=0.07) and increased with number of reported transfusions. Age-adjusted odds ratios (95% CIs) for HHV-8 seropositivity were 0.97 (0.54-1.75), 1.13 (0.59-2.17), 1.76 (0.81-3.83), and 2.17 (1.18-3.99) for children with 1, 2, 3, or 4-10 transfusions, respectively (p trend =0.007). We estimated that HHV-8 transmission risk was 2.6% per transfusion (95% CI 1.9-3.3). Secondary analyses based on the orf73 assay provided qualitatively similar results.

Conclusion: Our study suggests that HHV-8 can be transmitted by blood transfusion, albeit inefficiently. In Uganda, HHV-8 infection risk from blood transfusion is small compared to risks from community sources.
CELL DISTRIBUTION OF HERPES VIRUS 8 (KSHV/HHV8) DURING DEVELOPMENT OF KAPOSI'S SARCOMA LESIONS

P. Biberfeld1, P. Pyakurel1, C. Massambu1,3, E. E. Kaaya1,3, T. Heiden1, M. Enbom2, E. Heiden1. 1Immunopathology Lab., 2Swedish Institute for Infectious Disease Control, Karolinska Institutet, Stockholm, Sweden, 3Department of Pathology, Muhimbili University College of Health Sciences-MUCHS, Dar-es-Salaam, Tanzania.

Background: All clinical forms of Kaposi’s sarcoma (KS) are associated with the KS-associated herpes virus KSHV/HHV8, but the pathogenic mechanisms involved are not well understood. We have therefore analyzed the presence of KSHV/HHV8 in various cells of biopsies and serum of AIDS (AKS) and non-AIDS, endemic KS (EKS) patients at various stages of development.

Methods: Cells were studied for expression of HHV8 latent antigen (LANA) and cell markers by combined triple immunostaining and HHV8-DNA in serum by a quantitative RT-PCR assay. HIV-gag was detected by immunohistochemistry and ELISA.

Results: LANA+ cells varied with stage of AKS and EKS in relation to the number of CD34+ tumor spindle cells (SC). However, a fraction (35-45%) of CD34+ cells at late (nodular) and early (patch, plaque) stages were LANA-negative, suggesting a heterogeneity in this cell population with regard to infectivity. Furthermore, a minor population (≤5-10%) of LANA+ cells, in early KS stages were CD34-negative with an apparent leucocytic morphology. Proliferating Ki67+ cells were seen at all KS stages. These cycling cells were more frequent in AKS then EKS, often LANA-negative, and positive for CD34 or lymphocyte markers. No significant difference in the relative number of CD34+ LANA+ cells was seen in comparable AKS and EKS stages. The level of KSHV/HHV8 DNA in serum was moderately higher in AKS than EKS, and usually higher in males as compared to females. Higher serum virus DNA values were seen in early (patch-plaque), KS-stages. In healthy blood donors KSHV/HHV8 serum levels (20%) were significantly lower compared to KS patients. HIV was found in some macrophages at different stages of KS development.

Conclusions: KSHV/HHV8 in AKS and EKS biopsies is associated not only with SC, but also with other cells. The majority of CD34+ SC are LANA+ suggesting a selective advantage in viability for infected cells. However, CD34+/LANA− SC appear to be continuously recruited to KS lesions. The presence of significant levels of KSHV/HHV8 DNA in the serum of both KS and non-KS Tanzanians indicates a high risk for blood viral transmission. Infiltration of HIV infected macrophages suggests local direct (HIV-tat) and indirect effects of virus factors.
MULTIFACTORIAL ANALYSES OF SERUM MARKERS OF B CELL ACTIVATION PRIOR TO THE DEVELOPMENT OF AIDS-ASSOCIATED NON-HODGKIN’S B CELL LYMPHOMA

Elizabeth Crabb Breen\textsuperscript{1}, W. John Boscardin\textsuperscript{2}, Marta Epeldegui\textsuperscript{1}, Hua Guo\textsuperscript{2}, Roger Detels\textsuperscript{2}, and Otoniel Martínez-Maza\textsuperscript{1}. \textsuperscript{1}David Geffen School of Medicine, and \textsuperscript{2}School of Public Health, University of California, Los Angeles.

AIDS-associated non-Hodgkin’s B cell lymphoma (AIDS-NHL) is the second most common AIDS-associated malignancy, occurring in approximately 10% of all persons with AIDS. B cell hyperactivation is a characteristic feature of HIV infection, and is thought to contribute to the greater than 70-fold increase in risk for NHL among persons with AIDS compared to the general U.S. population. Utilizing archived serum samples from the Multicenter AIDS Cohort Study (MACS), which has followed homosexual men at six-month intervals since the early 1980s, we have been able to assess serum levels of various markers of B cell activation in samples obtained as close as possible, but prior to the clinical diagnosis of AIDS-NHL (range 1-31 months preceding lymphoma, median = 5 months). We have previously reported that elevated levels of soluble CD27 (sCD27), IgE, sCD23, interleukin 6 (IL6), and IL10 are seen in the serum of men who go on to develop lymphoma, compared to men with an AIDS diagnosis, but no malignancy. We have also seen higher levels of sCD44 (p=0.01) and sCD30 (p=0.02), and lower levels of IgM and IgG (p=0.001) in serum preceding lymphoma, but no differences in serum IgA. Using logistic regression to consider multiple markers, we have examined our ability to predict whether a given individual is a lymphoma case (n=50) or AIDS control (n=44), and what markers go into that prediction. A four-predictor model suggests that elevated sCD23 and sCD27 and decreased IgM and IgG may be discriminatory for lymphoma (p≤0.03 each predictor when taking other three predictors into account); further multifactorial analyses including sCD30 and sCD44 are underway.
A NOVEL STRATEGY OF VIRAL IMMUNE EVASION BY KSHV ORF45

Fan Xiu Zhu and Yan Yuan. Department of Microbiology, University of Pennsylvania School of Dental Medicine, Philadelphia, Pennsylvania 19104, USA

ORF45 is an immediate-early protein of Kaposi’s sarcoma-associated herpesvirus (KSHV). Recently, we found that ORF45 interacts with cellular interferon regulatory factor-7 (IRF-7) and prevents IRF-7 from being phosphorylated and transported from the cytoplasm to the nucleus in response to viral infection. IRF-7 is a transcription regulator which plays a critical role in virus-mediated induction of interferon alpha (IFNA) gene expression. By blocking the nuclear translocation of IRF-7, ORF-45 efficiently inhibits the activation of type I interferon (α and β) genes during viral infection. However, its role in virus life cycle has not been addressed. Here we provide evidences to show that ORF45 is a virion-associated protein: 1) ORF45 can be detected in ultracentrifuge pellet of TPA induced BCBL-1 culture medium as well as gradient-purified virion lysate. 2) In a gradient centrifugation analysis, ORF45 peaked in the same fractions with KSHV virion. 3) Immuno-gold electron-microscopy study shows that ORF45 can be seen in virion-like structure in TPA induced BCBL-1 cells and purified virions. The presence of ORF45 in KSHV virion raised a possibility that this protein is delivered to host cells at the start of infection and blocks the establishment of antiviral state of host cells at very early stage of the infection, suggesting an important role of ORF45 in KSHV primary infection.
RITUXIMAB FOR HIV-ASSOCIATED CASTLEMAN’S DISEASE
AG Marcelin¹, L Aaron², C Mateus³, F Dreyfus³, JP Viard², V Calvez¹, N Dupin³, ¹GH Pitié-Salpêtrière, ²Hôpital Necker, ³Hôpital Cochin-Tarnier, Paris.

Background: KSHV infection is associated with Kaposi’s sarcoma (KS) and Castleman’s disease (CD). HAART has dramatically improved the prognosis of KS while it has little effect on the progression of CD. Treatment of CD is still a major problem for physicians in charge of HIV infected patients. Morphologic studies have shown that in CD lymph node biopsies, KSHV is present in IgM/lambda restricted plasmablast of the mantle zone with a variable expression of CD20. The aim of this study was to evaluate the benefit of rituximab on the evolution of CD in the setting of HIV infection.

Methods: Five HIV-1 positive patients with CD were included prospectively in a non randomized study evaluating the effect of 4 infusions of rituximab (350 mg/m² per week for 4 weeks). Evaluation was performed on clinical parameters (fever, lymph node enlargement, hepato-splenomegaly) and biological parameters (plasma HIV-1 RNA load, blood CD4/CD8, CD19 cell counts, c-reactive protein). KSHV viral load in PBMCs was evaluated before and sequentially after rituximab infusions using real time PCR (TaqMan®).

Results: Three out of 5 patients were in complete remission without any clinical symptoms with a follow up of 4 to 12 months after the last infusion of rituximab. Clinical remission was correlated with biological response, i.e. regression of inflammatory syndrome and significant decrease of KSHV viremia. Two patients died very quickly after the beginning of treatment with rituximab. In this 2 patients severe auto-immune disorders were associated with CD and rituximab had no effect on the CD19 cell count while the 3 responders experienced a dramatic decrease of the CD19 cell count following rituximab infusions.

Conclusions: Rituximab may represent a good treatment for the management of HIV-associated CD with a long term effect on both clinical and biological parameters. However it may be active only in a subset of patients free of auto-immune and/or hematological disorders associated with CD. The lack of blood B cell decrease in non responders may reflect « resistance » to rituximab. Our study confirms the clinical interest of monitoring KSHV viremia in patients with CD as it appears a good marker of disease progression and of therapeutic response.
Splenectomy is an Effective Treatment for HIV-Associated Castleman’s Disease. Paul Coty, Alan Astrow, David Gallinson, Grace Tarabay, Gerardo Capo, Zujun Li, William Cook, Department of Medicine and Comprehensive Cancer Center, St Vincent’s Catholic Medical Center-Manhattan, NY, NY.

We have reviewed our recent experience in treating HIV-associated Castleman’s disease (HIV-CD), a polyclonal lymphoproliferative disorder, in order to determine the role for splenectomy and the benefit from systemic chemotherapy. 18 patients were diagnosed with HIV-CD at our institution from 1996-2002. 10/18 patients underwent splenectomy because of B symptoms and/or symptomatic cytopenias and in 5 of those 10, splenectomy was the only treatment. 14/18 were receiving HAART at the time of diagnosis. 16 were male, 2 female. Median age at diagnosis was 40 (range 21 to 68), median duration with HIV-CD, 31 months (range 1 to 75). Fever was the presenting symptom in 12, fatigue in 9 and night sweats in 3. The median CD4 count at diagnosis (available in 14/18) was 336 (range 64 to 1152) and viral load (available in 12/18) was 206 (range undetectable to 700,000). LDH (available in 11/18) was normal in all but two. 11/18 patients had Kaposi’s sarcoma (KS) either cutaneously or as a small focus in the pathology specimen. Three specimens tested by PCR for HHV8 were positive. 10/10 patients responded to splenectomy with resolution of fevers and/or improvement in cytopenias. Steroids did not appear useful. Four patients received steroids as initial treatment. Three had no response and one had a transient response but then required splenectomy. Foscarnet also appeared to be ineffective with no response in five treated patients. Chemotherapy with doxil, daunosome, or the combination of cyclophosphamide, dexamethasone, plus doxil or daunoxome was given to six patients, five of whom had developed recurrent fevers and/or lymphadenopathy or progressive KS after splenectomy. All had symptomatic improvement in fevers, lymphadenopathy or KS. Two patients died of unknown causes. Our retrospective review suggests that splenectomy is an effective treatment for symptomatic HIV-CD and that systemic chemotherapy with Doxil or daunoxome may be effective for symptomatic lymphadenopathy and/or KS after splenectomy. Prospective study is needed to better define the clinical syndrome of HIV-CD and to determine the optimal treatment.
REMISSION OF HHV-8 AND HIV-ASSOCIATED MULTICENTRIC CASTLEMAN’S DISEASE WITH GANCICLOVIR TREATMENT.

Corey Casper\textsuperscript{1,2}, W. Garrett Nichols\textsuperscript{1,2}, Meei-Li Huang\textsuperscript{2}, Anna Wald\textsuperscript{1}, Lawrence Corey\textsuperscript{1,2}.

\textsuperscript{1}University of Washington, and \textsuperscript{2}Fred Hutchinson Cancer Research Center.

\textit{Introduction:} Multicentric Castleman’s Disease (MCD) is a lymphoproliferative disorder associated with human herpesvirus 8 (HHV-8) infection among persons with Human Immunodeficiency Virus (HIV). Treatment often includes chemotherapy, and progression to non-Hodgkin’s lymphoma frequently occurs. MCD is characterized by active HHV-8 replication, and many of the symptoms of MCD may be attributable to viral gene products. We describe the effect of ganciclovir on the clinical and virologic course of MCD.

\textit{Methods:} Two HIV and HHV-8 co-infected persons with MCD were followed with clinical assessments, plasma HHV-8 DNA, HIV RNA, CD4 count, C-reactive protein, and interleukin-6 quantity.

\textit{Results:} Patient 1 initially was treated with intravenous acyclovir, 10 mg/kg thrice daily, with no effect on symptoms or detection of HHV-8 DNA. Oral (1500 mg thrice daily) and intravenous (5 mg/kg twice daily) ganciclovir resulted in a reduction in the frequency of episodic flares of MCD and detectable HHV-8 DNA. Oral valganciclovir, 900 mg twice daily for two weeks followed by once daily for 4 weeks, led to a lasting remission of MCD symptoms and HHV-8 viremia. Patient 2 initially was treated with oral valacyclovir, 2 grams twice daily, with no effect on symptoms or HHV-8 DNA detection. A six-week course of valganciclovir, as administered to Patient 1, subsequently was given. No additional flares of MCD or detectable HHV-8 viremia have occurred since that time.

\textit{Conclusions:} Ganciclovir may be effective in the treatment of HIV-associated MCD.
Role of KSHV Encoded vIRF-3/LANA 2 in AIDS Lymphogenesis

Barbora Lubyova, J. Augusto Frisancho, Karen E. Pinder, Betsy J. Barnes and Paula M. Pitha. Sydney Kimmel Comprehensive Cancer Center, Johns Hopkins School of Medicine, Baltimore, MD.

KSHV is associated with two major malignancies in AIDS patients, Kaposi’s sarcoma and B cell lymphoma- primary effusion lymphoma (PEL). The PEL cells are positive for KSHV and rarely carry mutated ras or p53 genes. The cell lines established from AIDS-PEL constitutively expressed only five viral genes vFLIP, vCYC, LANA-1, vIRF-2 and vIRF-3/LANA2. The cluster of four ORF within the 83-95 Kb region of KSHV shows homology to the cellular transcription factor of IRF family. Three of these vIRFs were cloned and characterized. The cellular IRF genes encode DNA binding proteins involved in the innate response to viral infection as well as differentiation of lymphoid cell, cell growth regulation and apoptosis. The KSHV encoded vIRFs target the cellular IRFs to eliminate the innate immune response and inhibit the cellular function of IRFs. KSHV encoded vIRF-3 encodes nuclear protein of 566 aa that is expressed in the latent stages of KSHV infection and shows close similarity to IRF-4. Interestingly co-cultivation of PEL cells with HIV-1 infected T cells results in an increase of vIRF-3 expression. In the present study two mechanisms of the pro-oncogenic potential of vIRF-3 were examined. First, we have observed that vIRF-3 binds to the cellular IRF-1, IRF-3, IRF-5 and IRF-7 and modulates their transcriptional activity. Both IRF-1 and IRF-5 were shown to be a tumor growth suppressing and proapoptotic genes and thus the question how the association of vIRF-3 with these two cellular IRFs modulates their growth regulatory and proapoptotic function as well as tumor suppressing effect has been addressed. Second, we have observed that vIRF-3 associates with tumor suppressor, c-myc-modulator-1 (MM-1). This 167 aa long protein contains a putative leucine zipper motif and is expressed preferentially in nucleus. MM-1 belongs to a group of molecular chaperons-prefoldins and suppresses transcription activity of c-myc and expression of c-myc targeted genes. By using specific vIRF-3 and MM-1 antibodies we detected the association of vIRF-3 and MM-1 in PEL cells. The effect of vIRF-3 and the association of MM-1 and c-myc and activation of c-myc targeted promoters, including cyclin dependant kinase, cdk4, is being examined. The results of these studies will be presented.
PROFILING KSHV/HHV-8 TRANSCRIPTION IN KAPOSI'S SARCOMA LESIONS: A DIRECT ROLE FOR LANA IN TUMORIGENESIS?

Department of Microbiology and Immunology, The University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104 e-mail: dirk-dittmer@ouhsc.edu

Kaposi’s sarcoma (KS) is the signature pathology of AIDS. Kaposi’s sarcoma-associated herpesvirus (KSHV/HHV-8) is causally linked to KS. Here, we report the first KSHV transcription profile in primary KS lesions using a novel, real-time PCR array. The KSHV latency I mRNAs encoding LANA/orf73, v-cyclin/orf72 and v-FLIP/orf71 were invariably present in KS, but viral lytic mRNAs were only detectable in a fraction of samples. Interestingly, the viral interferon regulatory factor 1 (vIRF-1/K9) clustered with LANA, but not its homolog vIRF-3/LANA-2, which is transcribed only in KSHV-associated lymphomas. This argues that vIRFs foster latent viral persistence, and that their redundancy may—in part—be explained by tissue-specific regulation (This study is supported by the AMC and uses material from the ACSB).

To investigate the transforming potential of LANA in vivo, we generated transgenic mice using the authentic viral promoter to direct B-lineage specific expression. These mice develop follicular hyperplasia, lymphoma and hepatic dysplasia. Our in vivo model establishes LANA as the principal oncogene of KSHV, and suggests a novel target for therapeutic agents against KS and KSHV-associated lymphoma.

The unusual set of clinical and biologic features of primary effusion lymphoma (PEL) coupled with consistent infection with KSHV suggest a lymphomagenic role of the virus in these lymphomas. Previous findings from our lab have demonstrated that constitutive activation of NF-κB is essential for the survival of KSHV-infected PEL cells. KSHV encodes a number of genes with signaling potential that have been reported to activate NF-κB when transfected into various cell lines, but we have found that of these only vFLIP, encoded by ORF71/K13, is expressed at levels that correlate with constitutive NF-κB activity in PEL cell lines. We have found that in human B lymphoma cells vFLIP activates NF-κB more efficiently than cellular FLIP homologs and induces expression of NF-κB-regulated anti-apoptotic genes. We also evaluated the involvement of vFLIP in the constitutive activation of NF-κB in KSHV-infected PEL cells by RNA interference. Using this approach, we were able to inhibit vFLIP protein production, which subsequently resulted in abrogated NF-κB activation, and down-regulation of NF-κB-dependent antiapoptotic genes. Moreover vFLIP siRNA treatment induced apoptosis of PEL cells and further sensitized them to extrinsic apoptotic stimuli. These results suggest that KSHV vFLIP has functional homology to EBV LMP-1 and HTLV-1 Tax in terms of its ability to activate NF-κB and promote survival of infected lymphoma cells. They also provide the first direct demonstration that a KSHV-encoded gene is essential for the survival of naturally-infected tumor cells.
HUMAN PAPILLOMAVIRUS E2 PROTEINS ASSOCIATE WITH MITOTIC SPINDLES: A NOVEL MECHANISM FOR VIRAL PERSISTENCE. Brian A. Van Tine, Luan Dao, Timothy M. Sonbuckner, Biing-Yuan Lin, Thomas R. Broker and Louise T. Chow. Department of Biochemistry and Molecular Genetics, University of Alabama at Birmingham, Birmingham, AL 35294-0005

Human papillomaviruses establish persistent infections in squamous epithelium. The circular, double-stranded viral DNA of about 8 kbp is maintained as extrachromosomal DNA at a low copy number in the nucleus of undifferentiated cells. Productive infection only occurs in cells undergoing terminal differentiation. Viral DNA replication requires virus-encoded E1 and E2 proteins. E2 is the origin recognition protein which recruits the viral helicase E1 to the ori. In turn, E1 recruits the host DNA replication machinery to initiate replication from the viral origin. The bovine papillomavirus E2 has been reported to associate with mitotic chromosomes. However, the mechanism by HPV DNA persists in infected cells has not been elucidated. We have constructed replication-competent E2 proteins, each fused at its amino terminus to the GFP protein. We were able to track the viral E2 proteins in transiently transfected cells. Our results showed that GFP-E2 proteins associated with mitotic spindles and centrosomes, as established by colocalization with α and β tubulins, respectively. Viral origin-containing DNA also associated with the spindles. Furthermore, HPV E2 mutated in the nuclear localization signal associated with microtubules and microtubule organizing center (MTOC, i.e., centrosome) during interphase. This conclusion was substantiated in a microtubule dissociation / recovery experiment when cells chilled to 4°C were warmed back to 37°C. The E2 domain responsible for this association has been delineated. These results demonstrate that HPV has a novel mechanism in maintaining persistence. They also suggest a model for destabilization of cellular chromosomes after integration of HPV DNA based on the breakage-fusion bridge cycle.
INHIBITION OF EBV IMMORTALIZATION: EFFICACY OF AN ANTI-EBV PEPTIDE

Christopher Farrell\textsuperscript{1}, Marek Cebrat\textsuperscript{1}, Philip Cole\textsuperscript{1}, and S. Diane Hayward\textsuperscript{1,2}

\textsuperscript{1}Dept. of Pharmacology and Molecular Sciences, \textsuperscript{2}Kimmel Comprehensive Cancer Center, Johns Hopkins School of Medicine, Baltimore MD

The Epstein Barr virus (EBV) EBNA2 protein is a transcriptional activator that is essential for the immortalization of B cells. EBNA2 binds to target genes through its interaction with the cellular DNA binding protein CBF1 and is known to induce changes in cellular and viral gene expression. Disruption of this interaction with a synthetic peptide that mimics the interaction domain of EBNA2 has been shown to block the association of EBNA2 and CBF1 in an \textit{in vitro} EMSA assay.

To extend these findings to a tissue culture model of EBV immortalization, the EBNA2 peptide was modified to allow membrane trafficking. The protein transduction domain of the HIV tat protein was fused to the EBNA2 peptide to allow the peptide to cross the cell membrane. The EBNA2-tat peptide retained the ability to block the EBNA2-CBF1 interaction as measured by a GST-pull down assay. Labeling with fluorescein confirmed that the EBNA2-tat peptide efficiently entered B cells. The peptide had a half-life of about 24hrs. Peptide treated EBV immortalized B cell lines (LCLs) showed reduced cell growth and viability whereas EBV negative B cells (DG75) did not. In an \textit{in vitro} immortalization assay, the EBNA2-tat peptide treatment blocked the ability of EBV to immortalize B cells. A control peptide had no effect.

In conclusion, inhibition of the EBNA2-CBF1 interaction by an EBNA2-tat peptide is able to prevent immortalization of B cells and interfere with proliferation of established EBV\textsuperscript{+} B cell lines.
INHIBITION OF REPLICATION MACHINERY BLOCKS REPLICATION OF EBV-BASED PLASMIDS

Nikhil Wagle, Suman K. Dhar, Sandeep Saxena, Yuichi Machida and Anindya Dutta. Dept. of Pathology, Brigham and Women’s Hospital, Harvard Medical School, Boston, MA 02115.

INTRODUCTION: Epstein-Barr virus (EBV) latently infects B cells or epithelial cells in 90% of humans and can cause malignancies in immunosuppressed patients. OriP, a region of the EBV chromosome, supports the replication of recombinant plasmids in human cells in the presence of the EBV-encoded protein EBNA-1. Plasmids bearing oriP are replicated once per cell cycle, suggesting that oriP is controlled by the cellular replication machinery. This machinery includes ORC and MCM2-7, proteins that are required for firing of chromosomal replication origins, and geminin, a protein that suppresses inappropriate firing of origins. Our aim was to examine the machinery necessary for latent replication of EBV and to determine if alterations in this machinery could be used to selectively inhibit replication of EBV-based episomes. METHODS: We created a cell-line that is partially defective in ORC and another cell-line that overexpresses geminin to test whether the cellular replication initiation apparatus is required by EBV-based episomes in cancer cells. RESULTS: A hypomorphic mutation in ORC2 impaired replication from oriP. Overexpression of geminin also prevented oriP replication without affecting chromosomal origins. Similar results were obtained in experiments using RNA interference directed against EBNA-1. Both ORC and MCM2-7 interact with oriP, and Orc2 physically interacts with EBNA-1, suggesting that EBNA-1 recruits the cellular replication machinery to oriP. CONCLUSIONS: ORC is essential for replication initiation from oriP of EBV. Inhibition of replication initiation factors, by mutation of ORC, over-expression of geminin, or RNAi against EBNA-1, can selectively inhibit EBV-based episomes. Agents that inhibit the cellular replication initiation apparatus may be useful for clearing latent infection by EBV for both the therapy and prevention of EBV-induced lymphomas or nasopharyngeal carcinomas in patients with AIDS and other immunosuppressive conditions.
RNA INTERFERENCE OF HPV18 E6 AND E7 INDUCES SENESCENCE IN HELA CELLS
Allison H.S. Hall¹ and Kenneth A. Alexander¹,²
¹Department of Molecular Genetics and Microbiology,
²Department of Pediatrics
Duke University Medical Center, Durham, North Carolina

The human papillomavirus (HPV) oncoproteins E6 and E7 promote cell proliferation and contribute to carcinogenesis by interfering with the activities of p53 and pRb, respectively. Previous work has shown that using the papillomavirus transcriptional regulator E2 to repress E6 and E7 expression in HPV-transformed cells inhibits cell growth by inducing apoptosis or senescence. However, as E2 appears to have effects on cells that are independent of E6 and E7, the specific effects of reducing E6 and E7 expression are not clear. We used RNA interference to directly reduce expression of E6 and E7 in HeLa cells (an HPV18-transformed cell line) without introducing additional proteins. A small interfering RNA molecule targeted to the E7 region of the bicistronic E6/E7 mRNA was used to induce RNA interference. We found that this small interfering RNA significantly reduced expression of E6 and E7. RNA interference of E6 and E7 also inhibited cellular DNA synthesis and induced morphologic and biochemical changes characteristic of cellular replicative senescence. These results demonstrate that reducing E6 and E7 expression alone is sufficient to cause HeLa cells to become senescent.
AIDS-RELATED KAPOSI'S SARCOMA: IMATINAB MESYLATE-INDUCED REGRESSION IN C-KIT POSITIVE DISEASE

Henry Koon, Glenn Bubley, Liron Pantanowitz, David Masiello, JoAnn Proper, Will Weeden, Brad Smith, Katherine Crosby, Steven R Tahan, and Bruce J Dezube; Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215.

PURPOSE: Human herpesvirus-8 (HHV8) expression of c-kit receptors promotes the growth and transformation of AIDS-related Kaposi’s sarcoma (KS) spindle and endothelial cells. It is unknown if this tyrosine kinase receptor is phosphorylated (activated) in KS. If it is activated, then the inhibition of the c-kit signal transduction pathway may provide a potential therapeutic target. We investigated the activation status of c-kit in KS lesions and evaluated the clinical response of KS to the c-kit inhibitor imatinib mesylate (Gleevec).

PATIENTS AND METHODS: Five male patients with AIDS-related cutaneous KS, which progressed despite chemotherapy \( [N=4, \text{average of } 3.5 \text{ prior therapies per patient}] \) and/or highly active antiretroviral therapy (HAART) \( [N=5] \), received imatinib mesylate administered orally, 300 mg twice daily. Dose reduction to 200 mg twice daily was permitted for toxicity. Response was determined by serial tumor measurement and in three individuals histological evaluation of a skin punch biopsy was obtained at baseline and following one month of therapy. Immunohistochemistry was performed using a phosphorylated and non-phosphorylated c-kit monoclonal antibody (Cell Signaling Inc. Beverly, MA).

RESULTS: All pre-imatinab KS lesions showed strong immunoreactivity in the endothelial and spindle cells for both phosphorylated and non-phosphorylated c-kit. Four of five patients had a partial response. In all three of the patients who were biopsied, histologic regression was documented (complete in one case and partial in two cases). During treatment, three patients had diarrhea (two grade 3 and one grade 4) and one had neutropenia (grade 3) warranting dose reduction.

CONCLUSIONS: Phosphorylated (activated) c-kit is expressed in AIDS-related KS. Imatinib mesylate administered orally twice daily for KS results in clinical and histologic regression of cutaneous lesions within one month following therapy. The success of this agent demonstrates that the c-kit receptor may provide a promising in vivo target for KS therapy.
HEPATITIS C VIRUS (HCV) AND B CELL LYMPHOMA (NHL): MECHANISMS OF ONCOGENESIS AND RESPONSE TO ANTI-HCV THERAPY

VMH Sung1, K Machida1, S Shimodaira1, MMC Lai1, AM Levine1. 1USC Keck School of Medicine, Los Angeles, CA

Background: HCV is associated with B cell NHL (Ann Int Med 127:423,1997). We established a B-NHL cell line (SB) persistently infected with HCV (J Virol 2003; 77: 134). The line produces HCV in culture which can infect primary human hepatocytes, PBMC's and a B cell line (Raji). We report details of anti-HCV therapy in pt SB, and show that HCV infection of B cells causes enhanced mutation of immunoglobulin and oncogenes.

Methods: Clinical data was reviewed. Raji cells were infected with HCV derived from SB cells. Control Raji cells were infected with UV-inactivated HCV. Multiple clones of Ig genes, p53, beta catenin and bcl-6 were sequenced and results compared between HCV+ and HCV- Raji cells.

Results: In 9/95, a 66 yr old male had HCV, cryoglobulinemia, and NHL in marrow (CD20+/CD5+). NHL progressed to the colon, now bcl-1+/CD20+/CD5-. Chemotherapy was ineffective. Splenectomy revealed mantle cell NHL (bcl-1+) with 2 distinct abnormal clones: t (11:14) and 12+. Peg-IFN + ribavirin was associated with sustained virologic response and pt has been in CR since 11/01. Raji cells infected with HCV from SB induced a 5- to 10-fold increase in mutation frequencies of p53, beta-catenin and bcl-6, confirmed by a plasmid reporter gene assay, revealing that HCV induced 2 types of mutation pathways involving: 1) somatic hypermutation of the Ig gene (with RGYW targeting preference, a higher GC/AT substitution and a higher replacement/silent mutation ratio); and 2) mutations of p53 and beta-catenin. HCV+ NHLs contained multiple amplified mutations in p53 and beta catenin genes, with nucleotide mutation patterns similar to the HCV infected B cells. Conclusions: (1) HCV infects B cells; (2) HCV induces a mutator phenotype, contributing to its potential oncogenesis; (3) HCV induced mutations of cellular genes are selected during development of B cell NHL; and (4) Rx aimed at HCV may be effective in treating NHL in these pts.
MULTI-MODALITY IMAGING TECHNIQUES TO ASSESS KAPOSI'S SARCOMA ASSOCIATED WITH ANGIOGENESIS


Kaposi sarcoma (KS) is a highly vascular tumor that is frequent course of morbidity and mortality among people with acquired immunodeficiency syndrome (AIDS). Angiogenesis can play a central role in the development and progression of KS. Currently, no non-invasive standard technique is available to assess the effect of anti-angiogenesis base therapy on blood flow in KS. The purpose of this study is to investigate the applicability of two non-invasive methods for the assessment of vascularity and vascular changes associated with KS: infrared thermal imaging (thermography) and Laser Doppler imaging (LDI). Thermography provides a two-dimensional image of superficial skin temperatures. The concept is that higher temperatures occur in the skin superficial to veins that are involved in active transport of blood. LDI is a modality that we are using which produces two-dimensional images of blood velocity over a defined area. Nineteen patients have been investigated. A comparative image analysis of thermography, between the lesion and the adjacent area of lesion free sites, shows most of the lesions are warmer but few less intense cooler than the adjacent site. Similar relationships are also observed in laser Doppler images (p<0.05). We are planning to study more to understand these phenomena. The results indicate that the techniques may be useful to assess or monitor the effect of anti-angiogenesis bases drug therapy on blood flow in KS tissue.
ASSESSING THE EFFECTS OF FLT3 LIGAND AND INTERLEUKIN-2 ON LYMPHOPOIESIS IN VIVO

Martin Guimond, Aharon Freud, Charlene Mao, Javier Barbosa, Marta Cavalcanti, Megan Cooper, Michael A. Caligiuri

From the Division of Human Cancer Genetics and The Division of Hematology-Oncology, The James Cancer Hospital and Solove Research Institute, The Ohio State University Comprehensive Cancer Center, Columbus, OH 43210.

We are interested in cytokines that can modulate natural killer (NK) cell numbers in vivo, in an effort to understand how this innate immune effector cell can be manipulated in people with immune deficiencies in order to enhance antibody mediated killing and vaccine strategies. The administration of low dose interleukin-2 (IL-2) results in a selective expansion of NK cells in humans, while FLT3 ligand (FL) has similar properties in mice but not in humans. Here, we investigated the in vivo effects of FL and IL-2 in mice. Eight-week-old C57BL/6 mice were treated with subcutaneous pegylated IL-2 (1.5 x 10^4 IU/d) and intraperitoneal FL (10ug/d), IL-2 alone, FL alone, or vehicle alone for 14, 28 and 90 days. The daily injection of IL-2 alone did not result in a significant expansion of NK1.1+CD3- NK cells compared to mice treated with placebo. However used of FL led to a rapid expansion of NK cells in the spleen (P < 0.001), bone marrow (P < 0.001), and blood (P < 0.001) with insignificant difference in combination with IL-2. NK cytotoxicity and interferon gamma transcript was enhanced with FL+IL-2, but not with either cytokine alone. NK precursor frequency increased during treatment with IL-2, FL, and FL+IL-2. Injection of IL-15 “knockout” mice with IL-2+FL confirms that neither cytokine is able to restore the NK cell deficit. However IL-2 injection led to the expansion of a SCA-I+SCA-II+ population in the marrow (Ctl: 1.9%, IL-2: 8%, FL+IL-2: 10%); spleen (Ctl: 8.5, IL-2: 50%, FL+IL-2: 62%); and blood (Ctl 2%, IL-2: 59% and FL+IL-2: 63%). Moreover, administration of FL+IL-2 gave rise to a subset of SCA-I+c-kit+ cells in the marrow (Ctl: <1%, IL-2: <1%, FL+IL-2: 10%); spleen (Ctl: 2<1%, IL-2: <1%, FL+IL-2: 9.8%); and blood (Ctl: <1%, IL-2: <1%, FL+IL-2: 5%). These results suggest that FL and IL-2 may work together to increase the proportion of precursor cells committed to the NK cell lineage and indicate that their action likely occurs early in NK cell differentiation.
TELOMERE REPEAT BINDING FACTORS TRF1, TRF2, AND hRAP1 MODULATE REPLICATION OF EPSTEIN-BARR VIRUS ORIP

Zhong Deng¹, Constandache Atanasiu¹, Dominique Broccoli², and Paul M. Lieberman¹∗

The dyad symmetry region (DS) of Epstein-Barr virus contains four EBNA1 binding sites that are punctuated by nine base pair repeats referred to as nonamer sites. We have recently found that telomere repeat binding factor 2 (TRF2) binds to these nonamer sites in vivo and in vitro, and that mutation of these sites affects episomal maintenance of OriP. In this work, we further characterize the role of the nonamer repeats and their cognate binding proteins in both DNA replication and plasmid maintenance function of OriP. We found that substitution mutation of all three nonamer sites reduced both DNA replication and plasmid maintenance of OriP containing plasmids in Raji cells. The nonamer sites further enhanced plasmid maintenance when cells were grown under conditions of genotoxic and oxidative stress. The nonamers were required for stable binding of TRF1, TRF2, hRap1, and Tankyrase to the dyad symmetry element, but were not essential for the binding of EBNA1 or ORC2 as determined by DNA affinity purification from nuclear extracts. Chromatin immunoprecipitation assays indicated that TRF1, TRF2, and hRap1 bound OriP in vivo. Treatment of cells with hydrogen peroxide caused a loss of TRF1 and hRap1 binding to OriP. Overexpression of full length TRF1 inhibited OriP replication, and this inhibitory activity was dependent upon an intact myb DNA binding domain. In contrast, overexpression of full length TRF2 did not inhibit OriP replication. Dominant negative truncation mutants of TRF2 and hRap1 inhibited OriP replication. Knockdown experiments with siRNAs directed against TRF2 and hRap1 severely reduced OriP replication, while TRF1 siRNA did not inhibit OriP. These results indicate that TRF2 and hRap1 contribute positive modulating activities for OriP replication, while TRF1 opposes these factors and inhibits OriP replication. We propose that telomere repeat factors interact with DS in a dynamic manner to regulate OriP replication and plasmid maintenance activity.
25

INSERTION OF MYC INTO IGH ESTABLISHES NEW TRANSGENIC MOUSE MODEL OF HUMAN IMMUNODEFICIENCY-ASSOCIATED BURKITT LYMPHOMA t(8;14)(q24;q32) TRANSLOCATION

Siegfried Janz 1, Lino Tessarollo 2, Joong-Su Kim 1, James D. Owens 1, Seong-Su Han 1, Ted A. Torrey 3, Nicole McNeil 4, Thomas Ried 4, Herbert C. Morse III 3 and J. Frederic Mushinski 1

1 Laboratory of Genetics, NCI, 2 Mouse Cancer Genetics Program, NCI, 3 Laboratory of Immunopathology, NIAID, 4 Genetics Branch, NCI, NIH, Bethesda.

Purpose: Human immunodeficiency-associated Burkitt lymphoma (iBL) is an aggressive form of MYC-driven post-germinal center non-Hodgkin lymphoma that frequently develops in the context of severely impaired immune function occasioned by HIV infection. Accurate mouse models for iBL are needed to study the events involved in its initiation and progression and to test novel interventions. We decided, therefore, to generate a mouse model of the MYC-activating chromosomal t(8;14)(q24;q32) translocation that is widely believed to be the initiating event in the pathogenesis of iBL.

Experimental Procedure: We inserted a histidine-tagged mouse Myc gene (MycHis) into the mouse IgH locus just 5' of C and deleted the intronic heavy-chain enhancer, E. Result: The newly developed mouse strain, designated IgH-MycC, is the most accurate model of iBL t(8;14) available to date.

Conclusion: The important biological feature of the IgH-MycC gene insertion model is that Myc is deregulated in all B cells by the appropriate set of correctly spaced regulatory elements residing in the IgH locus. These features represent a significant advance over previously developed Myc transgenics, which rely on individual IgH control elements (e.g., E) to simply overexpress Myc in B cells.
ROLE OF HEPATITIS C VIRUS INFECTION IN MALIGNANT LYMPHOMA IN SPAIN
Silvia de Sanjose1,2, Alexandra Nieters3, James J Goedert2, Eva Domingo-Domenech1,4, Alberto Fernandez de Sevilla4, Ramon Bosch5, Pilar Herrera6, Alicia Domingo7, Jose Petit4, Xavier Bosch1, and Birgit Kallinowski8
1 Servei d’Epidemiologia &Registre del Cancer, Institut Catala d’Oncologia, Barcelona, Spain, 2 Viral Epidemiology Branch, National Cancer Institute, Bethesda MD, USA,3 Clinical Epidemiology German Cancer Research Center, Heidelberg, Germany, 4 Hematologia Oncologica, Institut Catala d’Oncologia, 5 Patologia, Hospital Verge de la Cinta, Tortosa, Spain, 6 Patologia, Ramon y Cajal, Madrid, Spain, 7 Patologia, Ciutat Sanitaria & Universitaria de Bellvitge, Barcelona, Spain, and 8 Dept of Internal Medicine IV, University Hospital of Heidelberg, Germany

Background: Hepatitis C virus (HCV) has been implicated in the etiology of malignant lymphomas. We estimated the risk of lymphoma associated with detection of HCV infection.

Methods: Cases (N= 531) were consecutive patients newly diagnosed with a lymphoid malignancy between 1998 and 2002 in four centers in Spain. Lymphomas were diagnosed and classified using the WHO Classification. Controls (N=600) were hospitalized patients matched to the cases by 5-year age group, gender and study center. Several medical conditions associated with severe immunosuppression precluded the eligibility of controls. Patients underwent a personal interview and blood sampling. HCV positive subjects were considered those with antibody response and/or detection of HCV RNA. Cases were systematically tested for HIV antibodies.

Results: HCV infection was detected in 40 cases (7.5%) and 23 (3.8%) control subjects. HCV was present in six of 16 HIV-related lymphomas and in four of eight organ-recipient-related lymphomas. HCV was associated with a two-fold increased risk for lymphoma [odds ratio (OR)= 2.06 95%CI= 1.21-3.51] as compared to HCV negative subjects. Among non HIV subjects the OR for all lymphomas was 1.75 (95%CI =1.01- 3.03) and among non-organ recipients the OR was 1.85(95%CI=1.07-3.18). Within all lymphoma categories, HCV was more common among diffuse lymphoma as compared to negative HCV (OR=4.13, 95%CI=1.93-8.83). This risk was reduced when HIV or organ allograft transplant status was controlled for (OR=2.3 95%CI= 0.90-5.64). A two-fold increased risk associated with HCV was also observed for marginal B-cell lymphomas and Hodgkin’s lymphomas irrespective of the immune status, but the ORs were not statistically significant.

Conclusions: Chronic infection with HCV is likely to play a role in the development of lymphomas. This association needs to be further explored in the context of severe immunosuppressive conditions.
CLINICO-PATHOLOGICAL CHARACTERISTICS OF GASTROINTESTINAL LYMPHOMAS ASSOCIATED WITH AIDS (GI-ARL) AND THE IMPACT OF HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART)

Sridhar Srinivasan¹, Kenichi Takeshita³, Beata Holkova¹, Kena Miller², Zale P Bernstein², Asher Chanan-Khan². ¹Depts of Medicine, State University of NY at Buffalo, ²Roswell Park Cancer Institute, Buffalo NY and ³Division of Hematology, New York University.

Introduction: In patients with AIDS associated lymphoma (ARL), GIT is a common extranodal disease site (17%-28%) with poor outcome (median survival of 4-6 months). HAART has changed the epidemiology of ARL in general but its impact on GI-ARL remains unknown. We conducted this study to determine the clinico-pathological characteristics and the effect of HAART on GI-ARL.

Method: We reviewed the tumor registry database of Bellevue Hospital and RPCI and identified all cases of ARL between 1990-2000. Of the 201 patients with ARL 37 (18%) had GI-ARL. These were divided into Group A [no-HAART (n=28)] and Group B [HAART (n=9)] and the incidence, histologic subtypes and the survival compared.

Results: Of the 37 GI-ARL patients, 28 (76%) were in group A vs. 9 (24%) in group B (incidence; 19% vs. 17% respectively). In group A and B high-grade histology was noted in 25 (89%) and 7 (78%) whereas advance stage disease was seen in 20 (71%) and 8 (89%) patients respectively. Median survival of all patients with GI-ARL was 7 months; whereas the median survival of Group A and B were 5 and 25 months respectively.

Conclusion: We noted a higher incidence of GI-ARL in males with a preponderance of hepatic involvement in the no-HAART group. In this study even though the total number of GI-ARL decreased after the introduction of HAART their incidence in relation to ARL remained unchanged. Interestingly the median survival of these patients was significantly prolonged (median 5 vs. 25 months) with the introduction of HAART. Our study shows a positive impact of HAART on the outcome of patients with GI-ARL.
PROSPECTIVE SCREENING FOR AIDS-RELATED NON-HODGKIN’S LYMPHOMA (AR-NHL) PATIENTS (PTS) IN EAST AFRICA AS PART OF A CLINICAL TRIAL

J. Orem, MW Otieno, C Banura, E Katongole-Mbidde, P Fu, J Johnson, A Ness, S Reynolds, J Black, E Feigal, C Whalen and SC Remick. 1Makerere Univ. Medical School and Uganda Cancer Institute, Kampala, Uganda; 2Univ. of Nairobi College of Health Sciences and Kenyatta National Hospital, Nairobi, Kenya; 3Comprehensive Cancer Center and 4Center for AIDS Research at CWRU and Univ. Hosp. of Cleveland, OH; and the 5NCI, Bethesda, MD, USA.

In East Africa there are clinical data for AR-NHL derived primarily from limited cancer registry, autopsy and case-control series (AIDS 14:2929, 2000; Int J Cancer 92:687, 2001). There are no prospective data on the clinical presentation of pts thought to have AR-NHL for consideration of enrollment on a clinical trial (see accompanying abstract Otieno et al.). We report herein our experience to date on 108 (Uganda 49, Kenya 59) consecutively screened pts between Feb 2001 and Dec 2002 for participation in a pilot phase II study. Of these pts, 96 (Uganda 38, Kenya 58) had confirmed NHL. A total of 12 (11 Uganda) pts were excluded since diagnostic biopsy did not confirm NHL (non-specific / MTb lymphadenitis 7/1; other malignancy 2/Hodgkin’s dis 1; and abscess 1). Of the NHL pts 54 (Uganda 14, Kenya 40) were HIV+ (56%). A total of 22 pts (Uganda 8, Kenya 14) were enrolled for a recruitment rate of 41% among HIV+/NHL+ pts. Reasons for non-enrollment included: PS=4 (n=18), CNS disease 6 (all Kenya), refusal to give consent (n=6; 3 each site), and other (n=2). There is a clear predominance of males who are HIV-negative with NHL (p=0.004); comparable numbers of men and women were diagnosed with AR-NHL. At presentation there was no significant clinical difference of NHL pts by age, sex, and grade between Ugandans and Kenyans. There was a significant difference between Kenyans and Ugandans in that there was a greater HIV prevalence (p=0.002), more advanced stage of disease (p=0.004), and PS ≥ 3 (p<0.0001) in Kenyan NHL pts. In summary, the protocol recruitment rate is ample but factors that limit participation include: advanced stage disease and poor PS, which reflect rapid disease progression in the setting of HIV-1 disease; and pts declining consent. [This project is supported in part by NIH grants nos.: CA83528, TW0001 and AI36219.]
PRELIMINARY OBSERVATIONS USING DOSE-MODIFIED ORAL COMBINATION CHEMOTHERAPY (DMOCC) FOR AIDS-RELATED NON-HODGKIN'S LYMPHOMA (AR-NHL) IN EAST AFRICA: TOXICITY & VITAL STATUS. MW Otieno¹, J Orem², C Banura², E Katongole-Mbidde², J Johnson³, A Ness⁴, S Reynolds⁴, J Black⁵, E Feigal⁵, P Fu⁴, C Whalen³ and SC Remick³⁴. ¹Univ.of Nairobi College of Health Sciences and Kenyatta National Hospital, Nairobi, Kenya; ²Makerere Univ. Medical School and Uganda Cancer Institute, Kampala, Uganda; ³Center for AIDS Research and ⁴Comprehensive Cancer Center at CWRU and Univ. Hosp. of Cleveland, OH; and the ⁵NCI, Bethesda, MD, USA.

The incidence of AR-NHL is increasing in East Africa. Clinical trial data are available to develop pragmatic therapeutic strategies to treat AR-NHL in regions of the world with the greatest burden of HIV disease (JNCI 94:718,2002). We have embarked on a pilot feasibility study administering DMOCC to patients (pts) with biopsy-proven AR-NHL in Uganda and Kenya. As of December 2002, 22 pts [Uganda – 8 (4M/4F) and Kenya 14 (6M/8F)] have been recruited to study (see companion abstract on screening – Orem et al.). Therapy consists of 2 cycles of therapy at 6-week intervals: CCNU 50 mg/m² is given on D#1 of cycle 1; etoposide 100 mg/m² D#1-3 each cycle; and cyclophosphamide and procarbazine each 100 mg/m² D#22-26 each cycle. Two pts were registered to study but did not receive chemotherapy because of rapidly fatal disease during screening. Median duration of follow-up is approximately 4 mos, range 0 – 19+ mos. Ten pts (50% treated pts) completed the full 2 cycles of therapy; 2 are in active therapy. Other than myelosuppression side effects have been limited. There have been 4 episodes of ≥ 3 myelotoxicity: with two episodes grade 3/4 febrile neutropenia with recovery (1 in each site) and 2 treatment-related deaths secondary to grade 4 myelotoxicity (1 in each site). Responses have been seen but are not confirmed at present. To date 12 pts have died; 10 pts are alive at 1+ to 19+ mos.; and 14% have survived 1 year. There are no excessive adverse events to date and there is evidence of disease activity. The feasibility for conducting a chemotherapy trial for a neoplastic complication of HIV-disease in this setting is manifest. [This project is supported in part by NIH grants nos.: CA83528, TW0001 and AI36219. Bristol-Myers Squibb and Sigma Tau Pharmaceuticals provided the chemotherapeutic agents for this trial.]
OUTCOME OF HIV-ASSOCIATED HODGKIN'S DISEASE (HIV-HD) IS IMPROVED BY HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART)


Background: There are only few data regarding the impact of HAART on the survival of pts. with HIV-HD. We analyzed the outcome of this population with respect to the use and efficacy of HAART, and to potential prognostic factors. Methods: Multicentric cohort study of pts. with HIV-HD diagnosed histologically between 1990-2002 in 9 German HIV centers. To evaluate overall survival (OS) and the effects of several variables on OS, Kaplan-Meier statistics and extended Cox regression analysis were performed. Response to ART was used as a time dependent variable and was defined as a CD4 increase of ≥100/µl and/or at least one viral load of <500 copies/ml during the first two years following diagnosis of HIV-HD. Results: We identified 57 pts. (six females) with HIV-HD, 22 pts. being still alive. Median age was 37.1 years. Median CD4 count at HD diagnosis was 166/µl, and 25 % had had a prior AIDS event. Although 78 % of the pts. achieved a complete remission (CR), OS was relatively poor with a median of 42 months. One-year-OS and two-year-OS were 78 % and 64 %, respectively. In the univariate analysis, CD4 counts > 100/µl, age < 45 years, and HAART response were associated with prolonged OS (p<0.05). In the extended Cox model (p<0.02 for entry), the only factors independently associated with OS were HAART response (RH 0.21; 95% CI 0.07-0.64), and an age < 45 years (RH 0.29; 95% CI 0.11-0.73). Median survival time in pts. without HAART response was 18.6 months (pts. with HAART response: 89 % OS at 24 months). Conclusions: In this large cohort of pts. with HIV-HD, a significant improvement in survival was found in those pts. responding to HAART.
AIDS-ASSOCIATED BURKITT- OR BURKITT-LIKE LYMPHOMA (BL/BLL) – ‘STANDARD’ CHEMOTHERAPY MAY NOT BE ENOUGH

C. Hoffmann¹, M. Corzillius¹, E. Wolf², G. Fätkenheuer³, A. Stoehr⁴, A. Plettenberg⁴, T. Lorenzen⁴, H.-J. Stellbrink⁵, J. van Lunzen⁵, H. Jäger², H.-A. Horst¹. ¹Univ. of Kiel, ³Cologne, ⁵Hamburg, ²K.I.S., Munich, ⁴Hosp. St. Georg, Hamburg, Germany.

Background: Outcome of HIV+ pts. with BL/BLL is usually poor with complete remission (CR) rates of 40-50 % and a median survival of < 1 year. We compared pts. receiving an intensified chemotherapy protocol with pts. treated with CHOP. Methods: Multicentric cohort study of pts. with ARL diagnosed between 1990-2002 in 7 German HIV centers. Protocol was adapted from the GMALL Study Group (B-NHL 90), consisting of six alternating cycles (3 x cycle A, 3 x cycle B) of polychemotherapy after a prephase treatment of cyclophosphamide (CP) and prednisone. During cycle A, VM26, Ifosphamide (IFO), Methotrexate (MTX), Cytarabine (Ara-C), Vincristine, and Dexamethasone are given. During cycle B, Ara-C, VM26 and IFO are replaced by doxorubicine and CP. Results: We identified 44 pts. with BL/BLL who received either CHOP (group A, n = 30) or the GMALL protocol (group B, n = 14). Median CD4 count at BL/BLL diagnosis was 164/µl, and 23 % had had a prior AIDS event. In group B, significantly more patients achieved CR (85 % vs. 37 %, p=0.0069, ITT-analysis). We also observed a trend for improved one-year survival in group B (67 % vs. 38 %, p=0.09). No treatment-related deaths were observed in group B, compared to 3 deaths in group A. However, in group B median CD4 count was significantly higher (229 vs. 90/µl, p=0.007) with more pts. receiving HAART after BL/BLL diagnosis (714 vs. 7/30, p=0.09). In multivariate analyses, the survival benefit of patients in group B was statistically not significant. Conclusions: The intensive GMALL-protocol may be feasible in HIV+ pts. with BL/BLL. Our data suggest that outcome is improved compared to pts. treated with “standard” CHOP chemotherapy. In the era of HAART, more intensive chemotherapy regimens should be considered in these pts.
32

PRIMARY EFFUSION LYMPHOMA (PEL) IN HIV-INFECTED PATIENTS (PTS): A MONOINSTITUTIONAL STUDY
Cecilia Simonelli, Michele Spina, Roberta Cinelli, Emanuela Vaccher, Renato Talamini, Ferdinando Martellotta, Giuseppe Vultaggio, Umberto Tirelli. National Cancer Institute, Aviano Italy.

Aim of the study was to describe the clinical feature and outcome of HIV-associated PEL and to compare these with the clinical feature and outcome of the other HIV-associated NHL, followed in our institution. From April 1987 to December 2001, 277 pts with HIV-infection and systemic NHL were diagnosed and treated at the Aviano Center. PEL was diagnosed in 11 (4%) pts: 10 male and 1 female belonging to the following risk group for HIV-infection: 7 homosexual, 3 intravenous drug users and 1 heterosexual; median age was 41 yrs (range 26-58); 11/11 were stage IV for NHL; primary site of NHL was pleural effusion in 7, peritoneal effusion in 3 and pericardium effusion in 1. Eight were treated with CHOP-like regimen, 2 did not receive any treatment and one received only HAART. Complete remission was reached in 42% of pts and median survival was 6 months. Clinical features and outcome of 11 PEL pts were compared with the following groups: 11 pts with PBLOC, 76 pts with IBL ALCL and 75 LNCCL pts. No significant statistically differences were observed in the demographic data between the four groups, except for a higher percentage of homosexual men in PEL vs LNCCL group (54% vs 11%, p=0.003). At the onset of NHL the PEL pts showed a higher rate of C3 HIV stage in comparison with the other 3 groups, whereas no significant differences were seen in CD4 cell count and in HIV-viraemia. No statistically significant differences were found in clinical features at the NHL presentation: PS, systemic symptoms, IPI, LDH level. On the contrary, PEL pts showed a worse outcome in comparison with LNCCL group: overall survival at 2-years was 36.6% vs 53.3%; p=0.001.

In conclusion, PEL is a rare HIV-NHL type occurring as a late manifestation of HIV-infection with a poor clinical outcome and a shorter overall survival in comparison with the LNCCL group.
EVALUATION OF SCORING SYSTEMS FOR PROGNOSIS OF AIDS-RELATED NON HODGKIN LYMPHOMA

C. Lüke1, J. Unseld1, K. Possinger1, M. Ruhnke1
1 Medizinische Klinik II m.S. Hämatologie/ Onkologie der Charite, Germany

Background: The association between HIV infection and the development of lymphoma has been known since the early days of AIDS. While the introduction of highly active antiretroviral therapy (HAART) was able to dramatically reduce the incidence of several HIV-related diseases like opportunistic infections and some malignancies (e.g. Kaposi-Sarkoma), the incidence of AIDS-related Non Hodgkin Lymphomas (AIDS-NHL) is steadily increasing.

For HIV-related NHL, the treatment strategies are still controversial and therefore prognostic indicators are particularly important.

The international prognostic index (IPI) was created in the 1980ies as an instrument to estimate the individual prognosis of patients with aggressive NHL to adjust therapy regimens. As the relevance of prognostic indices in HIV patients is not clear, we assessed the practicability of IPI, as well as, the adapted age-adjusted-IPI in this cohort.

Methods: Seventy-four patients with HIV-related systemic NHL, attended at a single institute over a 12 year period, were analysed retrospectively. Univariate and multivariate methods were used for statistical analysis.

Results (preliminary): According to IPI (age-adjusted IPI) 42 %(20%) of the evaluated patients were assigned to the low risk group, 20% (26%) to the low intermediate group, 32% (43%) to the high-intermediate group and 6% (11%) to the high risk group in the whole study population. The correlation of the initial estimation according to the IPI with the course of the disease was generally poor. Especially in the group of patients with short survival(≤ 10 month) the risk was underestimated: merely 12% of patients were initially classified for high risk group, but 25 % for low risk group. Nearly the same results were obtained for the age-adjusted IPI (Low: 3%, low intermediate: 25%, high intermediate: 50% high:22%)

Conclusion: The presented data indicate, that the International Prognostic Index for aggressive NHL as well as the age-adjusted IPI tends to underestimate risk in HIV-positive individuals. A modification for this group of patients might be reasonable.
PHARMACOLOGICAL CYCLIN DEPENDENT KINASE INHIBITORS INHIBIT IMMEDIATE EARLY GENE EXPRESSION OF HHV8

Elodie Ghedin\textsuperscript{1,2}, Anne Pumfery\textsuperscript{2}, Cynthia de la Fuente\textsuperscript{2}, Vincent LaCoste\textsuperscript{2}, Steve Jacobson\textsuperscript{3}, Fatah Kashanchi\textsuperscript{2}.

\textsuperscript{1}The Institute for Genomics Research, \textsuperscript{2}The George Washington University Medical Center, \textsuperscript{3}National Institute of Neurological Disorders and Stroke.

Microarray technology allows the simultaneous analysis of thousands of genes. Most genome arrays are designed to represent a single species and to define the gene expression patterns of the organism in different conditions. Because of the high-throughput quality of microarrays, this technology holds great potential for diagnosis and subtyping. We have developed a DNA ‘virus chip’ comprised of PCR products of open reading frames (ORFs) from HIV and viruses known to be secondary infections in AIDS patients, including HHV6A, HHV6B, HHV8, EBV, HCV, HTLV-1, and HTLV-2. PCR products of 30 human genes were arrayed as controls. All 82 HHV8 ORFs were printed. We validated the specificity of the virus chip for the viruses listed using genomic DNA and total RNA from infected cells lines and have begun to use the chip in HHV8 experimental studies. Following treatment of BCBL-1 cells with PMA, we demonstrated that at 9 hours post-treatment, expression of a number of immediate early genes were induced, including K5, K7, bZIP, K13, rta, vCyclin, and LANA. Furthermore, treatment of PMA-induced BCBL-1 cells with Roscovitine down-regulated expression of all induced genes. These results suggest that HHV8 may utilize cellular cyclin dependent kinases to activate viral gene expression similar to HIV-1. Furthermore, these results indicate that Roscovitine may be useful in treating HHV8-associated diseases. We will extend these studies by analyzing gene expression over the entire viral life cycle as well as utilizing other inhibitors of cyclin dependent kinases.
ENDOTHELIAL INFECTION WITH KSHV GENES IN VIVO REVEALS THAT VGPCR INITIATES KAPOSI’S SARCOMAGENESIS AND CAN PROMOTE THE TUMORIGENIC POTENTIAL OF VIRAL LATENT GENES

Silvia Montaner1*, Akrit Sodhi1,3*, Alfredo Molinolo1, Thomas H. Bugge2, Earl T. Sawai3, Yunsheng He4, Yi Li5, Patricio E. Ray4 and J. Silvio Gutkind1

1Cell Growth Regulation Section, Proteases and Tissue Remodeling Unit, Oral and Pharyngeal Cancer Branch, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD; 2University of California at Davis, Davis, CA; 3University of California at Davis, Davis, CA; 4Children’s National Medical Center and The George Washington University, Washington, DC; 5Memorial Sloan-Kettering Cancer Center, New York, NY

The Kaposi’s Sarcoma Herpesvirus (KSHV) has been identified as the etiologic agent of Kaposi’s Sarcoma (KS), but initial events leading to KS development remain unclear. Characterization of the KSHV genome reveals the presence of numerous potential oncogenes. To address their contribution to the initiation of the endothelial cell-derived KS tumor, we developed a novel transgenic mouse that enabled endothelial cell-specific infection in vivo using virus expressing candidate KSHV oncogenes. Here we show that transduction of one gene, vGPCR, was sufficient to initiate angioproliferative tumors that strikingly resembled human KS. Endothelial cells expressing vGPCR were further able to promote tumor formation by cells expressing KSHV latent genes, suggestive of a cooperative role among viral genes in the promotion of Kaposi’s Sarcomagenesis.
KSHV G PROTEIN-COUPLED RECEPTOR VGPCR IMMORTALIZES HUMAN ENDOTHELIAL CELLS BY ACTIVATION OF THE VEGF RECEPTOR-2/ KDR

C. Bais¹,²  A.Van Geelen¹,², P. Eroles¹,², A. Mutlu¹,², C. Chiozzini¹,³, S. Dias², R.L. Silverstein², S. Rafii² and E.A. Mesri¹,².

¹Laboratory of Viral Oncogenesis, ²Division of Hematology-Oncology. Dept. of Medicine, Weill Medical College of Cornell University, New York, NY, 10021. eamesri@med.cornell.edu

Expression of KSHV vGPCR (ORF 74) in vitro and in vivo can cause VEGF angiogenesis, cell transformation, and initiate KS sarcomagenesis. However, since it is a lytic gene not expressed in the majority of latently infected spindle cells of KS lesions his role in malignant transformation has been unclear. We studied the consequences of vGPCR expression in human primary endothelial cells, which is are KSHV-target cells thought to be progenitors of KS spindle malignant cells. We found that expression of vGPCR in human umbilical vein endothelial cells (HUVEC) led to immortalization with constitutive VEGF receptor-2/ KDR (KDR) expression and activation. vGPCR immortalization was associated with anti-senescence mediated by alternative lenghtening of telomeres and an anti-apoptotic response mediated by vGPCR constitutive signaling and autocrine KDR signaling leading to activation of PI3K/ AKT pathway. We found that in the presence of VEGF, this mechanism can sustain supression of signaling by the immortalizing gene. We conclude that vGPCR can cause an oncogenic immortalizing event and recapitulate aspects of the KS angiogenic phenotype in human endothelial cells, pointing to this gene as a pathogenic determinant of KSHV. Our observation that vGPCR immortalizes human endothelial cells with autocrine KDR activation demonstrates the oncogenicity of KSHV lytic gene expression in target cells, since it shows that vGPCR expression in human endothelium is pro-angiogenic and pre-neoplastic, pointing to vGPCR and to the VEGF-KDR axis as potential targets for KS therapy. Evidence indicating that shutdown of vGPCR signaling can be overcome by VEGF, a KS growth factor, provides a molecular explanation for the feasibility of a “hit and run” oncogenic event initiated by vGPCR, in the multistep endothelial oncogenesis process leading to KS.
REGULATION OF KSHV LATENT TO LYTIC SWITCH BY HISTONE MODIFICATION AND NUCLEOSOME REMODELING

Fang Lu\textsuperscript{1}, Jing Zhou\textsuperscript{1}, Andreas Wiedmer\textsuperscript{1}, Kevin Madden\textsuperscript{2}, Yan Yuan\textsuperscript{2}, and Paul M. Lieberman\textsuperscript{1,3}

\textsuperscript{1} The Wistar Institute, Philadelphia, PA 19104
\textsuperscript{2} University of Pennsylvania, Department of Microbiology, School of Dental Medicine, Philadelphia, PA 19104

The switch from latent to lytic infection of Kaposi’s Sarcoma (KS)-Associated Herpesvirus (KSHV) is initiated by the immediate early transcriptional activator protein Rta/ORF50. We examined the transcriptional regulation of the ORF50 core promoter in response to lytic cycle stimulation. We show that the ORF50 promoter that regulates Rta transcription is highly responsive to sodium butyrate (NaB) and trichostatin A (TSA), two chemicals known to inhibit histone deacetylases. The NaB and TSA response element in the ORF50 promoter was mapped to a GC-rich element near the transcriptional start site. We demonstrate that Sp1/Sp3 can bind to this site in vitro and in vivo. High and low resolution micrococcal nuclease mapping studies revealed that a stable nucleosome is positioned over the transcriptional initiation and the Sp1/3 binding sites. Stimulation with NaB or TSA increased histone acetylation of nucleosomes associated with transcriptional initiation site of the ORF50 gene, and led to an increase in micrococcal and restriction enzyme sensitivity at the transcription initiation site. We found that ORF50 is associated with several different histone deacetylase proteins (including HDAC1, 5, and 7) in latently infected cells. Overexpression of the CREB-binding protein (CBP) histone acetyltransferase (HAT) stimulated ORF50 transcription in a HAT dependent manner, suggesting that CBP recruitment to the ORF50 promoter can be an initiating event for transcription and viral reactivation. Together, these results indicate that KSHV latency is regulated by a stably positioned nucleosome at the transcriptional initiation site of ORF50 that can be derepressed by CBP -dependent acetylation and subsequent chromatin remodeling.
ACTIVATION OF KSHV K8.1 LATE PROMOTER ASSOCIATES WITH THE VIRAL DNA REPLICATION
Shuang Tang, Koji Yamanegi and Zhi-Ming Zheng
HIV and AIDS Malignancy Branch, Center for Cancer Research, NCI/NIH, Bethesda, MD, USA
Kaposi’s sarcoma-associated herpesvirus (KSHV) or human herpesvirus 8 (HHV8) is a human gamma herpesvirus. We have recently mapped transcription start site of the late K8.1 to nt 75901 in KSHV genome. To identify a putative K8.1 late promoter upstream of the start site, a dual luciferase (Luc) reporter system was developed in JSC-1 cells, a KSHV+ B cell line, in which the putative K8.1 promoter with various sizes in length was cloned upstream of the Luc gene and the activity of the resulting plasmids was examined in JSC-1 cells with or without butyrate induction, a common means to activate viral late gene expression and KSHV lytic cycle in KSHV+ B cells. Our results show that the putative promoter had no activity in uninduced JSC-1 cells, but could be activated by butyrate in JSC-1 cells, not in 293 cells and Raji cells (EBV+, KSHV-), indicating that the activity of the putative K8.1 late promoter was JSC-1 cell-specific. Further extensive mapping of the putative K8.1 late promoter led us to identify a promoter core with 23 bp in length, containing a 5= CG-rich and a 3= TA-rich region. The activity of the core in butyrate-induced JSC-1 cells was orientation- and TA-rich region-dependent and could be inhibited to approximately 70% by phosphonoacetic acid (PAA), a viral DNA polymerase inhibitor, suggesting that the activity of the putative K8.1 promoter depends upon the viral DNA replication. A KSHV lytic origin (OriLyt) of DNA replication was then inserted into a KSHV late promoter-Luc reporter vector. The activity of the K8.1 late promoter in this plasmid was found being significantly enhanced, along with plasmid DNA replication, in butyrate-induced JSC-1 cells and again was sensitive to viral DNA replication inhibitors, such as PAA and ganciclovir (GCV), further confirming that activation of the KSHV late promoter, like ones in other herpesvirus, strongly associates with KSHV DNA replication.
HIV-1 TAT STIMULATES PROTEIN KINASE B (PKB) AND INHIBITS ANOIKIS
Felipe Samaniego, Jiang Wei, Daniel Young and Suizhao Wang, Departments of Lymphoma/Myeloma and Clinical Cancer Prevention, U.T. MD Anderson Cancer Center, Houston, TX

Background. To date, there has been no convincing mechanism offered for the aggressive growth of Kaposi’s sarcoma in human immunodeficiency virus (HIV)-1 infection. Because HIV-1 infection, and not other forms of immune deficiency, lead to Kaposi’s sarcoma it is reasonable to hypothesize that HIV-1 has a role in inducing Kaposi’s sarcoma. HIV-1 Tat (trans activator of transcription) is implicated in inducing KS because it stimulates endothelial cell proliferation, and promotes the growth of tumors induced by inoculation of KS SLK (Kaposi’s sarcoma lesion derived cells) in Tat transgenic mice. Given the lack of knowledge of underlying mechanisms in Tat-related tumor development, we have studied Tat signaling and anoikis (cell detachment induced apoptosis). Methods. Cell signaling and anoikis were monitored after treatment of KS SLK cells with Tat86, Tat peptides (HIV AIDS Research and Reference Reagent Program) in polyHEMA (5 mg/ml) coated wells. Apoptosis was analyzed by scoring of cells with apoptosis morphology after staining with Hoechst 33342 dye or propidium iodide. Cells in suspension were treated with signaling inhibitors Wortmannin or LY294002 and analyzed for cell signaling and anoikis. At time intervals, the levels of PKB and phosphorylated PKB levels were monitored in immunoblots using anti-PKB and anti-PKB-473 antibodies. Kinase assays were performed with [γ32P]ATP and myelin basic protein on immunoprecipitants using anti-integrin linking kinase antibody.

Results. KS SLK cells placed in polyHEMA-coated wells showed that approximately 20% of the cells remained viable whereas cells seeded in RGD peptide-, Tat86-, or fibronectin-coated wells contained 80% viable cells. RGD treatment of cells combined with Wortmannin or LY294002 prevented the inhibition of anoikis. Cells treated with RGD peptide or Tat86 showed phosphorylation of PKB that peaked with 5 ng/ml of Tat86. Treatment of the cells with RGD peptide showed a time dependent phosphorylation of PKB that was maximal after 20 minutes. Tat86 and RGD peptide treated cells contained the highest level of [γ32P]ATP incorporation in kinase assay of anti-integrin linking kinase antibody. Conclusions. Tat86 and RGD peptide inhibits anoikis. Tat induced phosphorylation of PKB and stimulated ILK kinase activity. As PKB is a central regulator of cell cycling and apoptosis, Tat may operate through PKB activation to prevent anoikis as an important mechanism in HIV-1 associated KS.
HUMAN HERPESVIRUS 8 (HHV-8) K1 SUPPRESSES APOPTOSIS OF BJAB CELLS INDUCED BY ANTI-FAS ANTIBODY
Felipe Samaniego, Daniel Young, Suizhao Wang
University of Texas MD Anderson Cancer Center, Houston, TX.

Background: HHV-8 is associated with the development of primary effusion lymphoma (PEL) and Kaposi's sarcoma (KS). PEL-derived cell lines express K1 spontaneously and is highly expressed during lytic phase viral replication. K1 encodes for a transmembrane protein containing a short cytosolic tail with an immunoreceptor tyrosine-based activation motif (ITAM). K1 has been shown to aggregate and potentially interact with other membrane receptors. K1 induces activation of NF-κB and its expression in animal models produces lymphoma. Given its NF-κB inducing properties, we anticipate that K1 contributes to transformation, in part, by suppression of apoptosis.

Methods: To study the role of HHV8 K1 gene product, we generated K1 stably expressing cell line BJAB-K1 and vector alone cell line BJAB-XS into retrovirus vector pLXSN and packaging cell line PT67 NIH 3T3. A truncated construct directing expression of K1 minus ITAM (K1m) was also generated. Anti-Fas antibody was used to induce apoptosis and cells were monitored for membrane protein Annexin-V and morphological features of apoptosis. Apoptosis of BJAB-K1 and BJAB-XS cells was induced by anti-Fas antibody (CH-11). Apoptosis rates of cell lines were compared with Annexin-V-FITC/PI analysis and DAPI-based nuclear morphological analysis. C-FLIP, caspase 8 and fas were monitored by immunoblot analysis and flow cytometry.

Results: The stable cell lines BJAB-K1 and BJAB-XS were verified by PCR-based detection of K1 and vector DNA. Fas mediated apoptosis was suppressed by 30±6% in BJAB-K1 cells versus BJAB-XS in Annexin-V assay (p<0.001). Apoptosis in BJAB-K1 was also suppressed by 51±11% when analyzed by DAPI staining (p=0.002). K1 expressing cells show consistent lower levels of caspase 8 activation when stimulated with anti-fas antibody. The levels of fas and c-FLIP remain unchanged in BJAB-K1 and vector transfected cells and when stimulated with CH-11 antibody.

Conclusion: Our data indicates that the expression of HHV8 K1 can suppress anti-fas mediated apoptosis. The results suggest that K1 can participate the development of PEL and KS through prevention of apoptosis.
41
ANTIVIRAL MEDIATED APOPTOSIS IN PEL THROUGH ACTIVATION OF TRAIL AND INHIBITION OF NF-κB
Juan Carlos Ramos1, Subrata Ghosh1, Ngoc L. Toomey1, Lisa Cabral1, Nicole C. Shank1, Gerold Feuer2 and William J. Harrington, Jr1. 1University of Miami and 2SUNY Upstate Medical University.

Gamma herpesvirus associated lymphomas occur frequently in immune compromised patients. These tumors share a common dependence on constitutive NF-κB activity. We have demonstrated that Epstein Barr virus (EBV) associated Burkitt’s lymphoma (BL) and Human Herpes Virus Type 8 (HHV-8) associated Primary Effusion Lymphoma (PEL) undergo apoptosis upon culture with the thymidine analogue Azidothymidine (AZT) (EBV+ BL) or AZT plus Interferon alpha (IFN-α) (PEL). AZT/IFN-α markedly inhibited PEL development in a SCID mouse model and rapidly (within 4 days) induced a complete durable remission in a patient with PEL. The apoptotic mechanism in PEL is mediated by the death receptor ligand TRAIL. IFN-α alone is insufficient to induce substantial apoptosis in PEL, however AZT potentiates TRAIL signal transduction through inhibition of NF-κB. AZT also blocks nuclear localization of NF-κB in EBV+ BL. This effect is clearly distinct from other chemotherapeutic and antiviral agents that induce rather than block NF-κB in gamma herpesvirus lymphomas. AZT-MP, the predominant intracellular metabolite in these lymphomas, inhibits phosphorylation and degradation of IκB. AZT/IFN-α mediated apoptosis is overcome by constitutive expression and nuclear localization of p50 coupled to the HSV transactivator VP-16. These data suggest that antivirals should be investigated as therapeutic agents for gamma herpesvirus lymphomas.
MRAIL-DEPENDENT NF-κB ACTIVATION BY K-CYCLIN


K-cyclin, the cyclin D2 homolog encoded by human herpesvirus 8 (HHV8), has non-cell cycle-related transcriptional regulatory properties. Data from our laboratory demonstrates that K-cyclin/cdk6 complexes bind and phosphorylate p300, and by doing so activate NF-κB dependent transcription. This activation did not require the histone acetyl transferase activity of p300 because wild-type and HAT-negative p300 when overexpressed with K-cyclin both synergistically activated NF-κB dependent transcription. In contrast, expression of a dominant negative cdk6 protein inhibited this activation suggesting that ability of K-cyclin to activate NF-κB-dependent transcription is cdk-dependent. This is significant because through our lab’s work the constitutive activation of the NF-κB transcriptional pathway in HHV8-associated tumors can be accredited precisely to K-cyclin’s activity. K-cyclin contains a protein docking site near the amino terminus, termed the MRAIL motif. This conserved site allows cellular cyclins to interact with proteins containing RXL motifs.

We questioned whether the MRAIL motif was essential for the K-cyclin/p300 interaction and the subsequent activation of NF-κB-dependent transcription. To answer this question, using site-directed mutagenesis we generated alanine mutants of the amino acid residues comprising the MRAIL motif. We have found that the mutation of any single amino acid in the MRAIL motif is sufficient to disrupt the ability of K-cyclin to activate NF-κB-dependent transcription using an NF-κB luciferase reporter assay. Moreover, GST-binding assays demonstrate p300 does not bind to these mutants. We have also shown that double and triple alanine substitutions had no additive effect. Thus, the interaction of K-cyclin’s MRAIL motif and RXL motifs in p300 are essential for the activation of NF-κB-dependent transcriptional activation. Such mutants will be useful in future studies to identify unique cellular or viral proteins that for phosphorylation substrates of K-cyclin/cdk complex that play a role in the pathogenesis of HHV-8.
KAPOSI’S SARCOMA-ASSOCIATED HERPESVIRUS K-CYCLIN ACTS AS A TRANSCRIPTIONAL REGULATOR WHEN TETHERED TO DNA. Kristy L. McDowell,1,2 Grant Westlake,2 Xiwu Chen,2 Christopher Magloire,3 and Philip J. Browning, M.D 2 1Meharry Medical College, Nashville, TN; 2Vanderbilt University, Vanderbilt-Ingram Cancer Center, Nashville, TN; and 3Taladega College, Taladega, AL. The Kaposi’s sarcoma-associated herpesvirus KSHV, also called human herpesvirus 8, encodes a homolog to cellular cyclin D2, K-cyclin. As with all cyclins, K-cyclin contains duplicated alpha helical repeats common to all cyclin repeats. Repeat I contains the core cyclin box through which cdk binding occurs. Repeat II has not been as well characterized but may play a role in protein-protein interactions. Our laboratory found that K-cyclin associates with transcriptionally active chromatin in KSHV infected cells. We therefore hypothesize that K-cyclin plays a role in the regulation of transcription through protein-protein and/or protein-DNA interactions. To study this, we generated a fusion protein consisting of the DNA binding domain of the yeast transactivator, GAL4 (GAL4BD) and K-cyclin. We examined the ability of this fusion protein to regulate transcription of a GAL4 DNA consensus reporter in Saccharomyces cerevisiae (S. cerevisiae) strain, Y187, and the mammalian cell line, 293H. In both Y187 and 293H cells, GAL4BD/K-cyclin activated transcription. We generated deletions of K-cyclin fused to GAL4DB to further characterize the activation domain. These mutants consisted of GAL4BD fusions of the first and second alpha helical repeats designated GAL4BD/K-cyclin Domain I and Domain II respectively. GAL4BD/K-cyclin Domain II activated transcription in contrast to GAL4BD/K-cyclin Domain I. These data suggests that K-cyclin, when tethered to DNA, activates transcription and that the transactivation domain resides in Domain II. Transient transfections were also performed with the GAL4BD/K-cyclin fusion, dominant-negative cdk-6 (DNcdk6) as well as p300 and p300ÅHAT plasmids. Transcriptional activation was doubled with p300 compared to K-cyclin alone and reduced with DNcdk6 and p300ÅHAT, suggesting that activation is cdk-dependent and requires the acetyltransferase activity of p300. Studies are ongoing studies in our laboratory to determine candidate proteins that may interact with K-cyclin to elicit such activation. Understanding how K-cyclin modulates transcription may expand our knowledge regarding its role in the pathogenesis of such diseases as Kaposi’s sarcoma and Primary Effusion Lymphoma.
VIRAL IRF-1 HAS A SHORT HALF LIFE AND TRANSIENTLY INHIBITS RESPONSES TO INTERFERON ALPHA DURING LYTIC REPLICATION OF HUMAN HERPESVIRUS 8 IN BCBL-1 CELLS

Veronika P. Pozharskaya, Laurie T. Krug and Margaret K. Offermann. Winship Cancer Institute, Emory University Medical School, Atlanta, GA.

Human herpesvirus 8 (HHV8), also known as Kaposi’s sarcoma (KS) herpesvirus, appears to be essential in the pathogenesis of KS and primary effusion lymphomas (PELs). Interferon alpha (IFNα) induces multiple changes in HHV8 infected cells despite HHV8-encoded genes that inhibit responses to IFNs, including viral interferon regulatory factor 1 (vIRF-1). The product of the vIRF-1 gene blocks responses to IFNs when overexpressed by transfection, yet the ability of vIRF-1 that is expressed during the course of viral infection to affect responses to IFNs has not been reported. We demonstrate that vIRF-1 is transiently expressed within the lytic phase of viral replication and has a half-life that is less than 2 hr. Most of the cells that express vIRF-1 also express DNA processivity factor (PPF or ORF59), yet PPF expression persists longer, leading to more cells within the lytic phase that express PPF than express vIRF-1, especially at later time points following lytic induction. Despite its short half-life, BCBL-1 cells that express vIRF-1 are resistant to some changes induced by IFNα, including expression of the double stranded RNA activated protein kinase. The level of expression of vIRF-1 is very low when cells are in the latent phase of viral replication or when vIRF-1 expression has declined after its initial induction during lytic viral replication. IFNα does not increase the amount of apoptosis that occurs in latently infected cells, whereas it effectively blocks the ability of HHV8 to enter the lytic cycle. Entry of BCBL-1 cells into the lytic phase of viral replication is accompanied by an increase in apoptosis. The amount of apoptosis is further enhanced if IFNα is added 48 hr following incubation with TPA, yet this has only a minor impact on viral production, suggesting that the decline in vIRF-1 occurs at a time when protection from IFNα-induced changes is no longer needed for successful production of virions.
THE KAPOSI’S SARCOMA-ASSOCIATED HERPESVIRUS (KSHV) LATENCY ASSOCIATED NUCLEAR ANTIGEN (LANA) INDUCES EXPRESSION OF ID-1, A CRITICAL CELL CYCLE REGULATORY PROTEIN, IN HUMAN ENDOTHELIAL CELLS. Jun Tang¹, Gabriel M Gordon¹, Maike Müller¹, Madhu Dahiya², Kimberly E Foreman¹. Loyola University Medical Center and ²Beth Israel Deaconess Medical Center.

The Kaposi’s sarcoma (KS)-associated herpesvirus (KSHV) is believed to be the etiologic agent responsible for KS. The pathogenesis of this potentially life-threatening neoplasm is complex and unclear, and it is currently unknown how KSHV causes KS. Id-1 (Inhibitor of DNA Binding-1 or Inhibitor of Differentiation-1) is a naturally occurring dominant negative inhibitor of basic helix-loop-helix transcription factors. Id-1 has been shown to inhibit cellular differentiation, promote cell cycle progression, and has recently been associated with tumorigenesis. In this report, we demonstrate that Id-1 is expressed at high levels in KS tumor cells both in vitro and in vivo, but is expressed at relatively low levels in endothelial cells (ECs), the likely precursor of the KS cell. KSHV infection of the progenitor cells may be responsible for Id-1 induction as KSHV infection of ECs in vitro results in a 27-fold increase, on average, in Id-1 protein expression under our experimental conditions. Furthermore, we demonstrate that the KSHV-LANA protein appears to be involved. Expression of LANA in ECs results in Id-1 induction similar to that seen in KSHV infected ECs. In contrast, KSHV encoded viral cyclin modestly induced Id-1 expression (average: 5-fold) and KSHV encoded viral FLIP had no effect. These results demonstrate the overexpression of Id-1 in KS cells and indicate the LANA protein may be responsible for Id-1 induction in infected EC. As Id-1 promotes cell cycle progression and has been associated with tumorigenesis in other systems, the induction of this protein by LANA may be an important mechanism by which KSHV allows infected ECs to escape normal cell cycle regulation ultimately leading to the development and/or progression of KS.
K-BZIP OF KAPOSI’S SARCOMA-ASSOCIATED HERPESVIRUS/HUMAN HERPESVIRUS TYPE 8 (KSHV/HHV-8) BINDS KSHV/HHV-8 RTA AND REPRESSIONS RTA-MEDIATED TRANSACTIVATION

Wei Liao¹, Yong Tang¹, Su-Fan Lin², Hsing-Jien Kung² and Chou-Zen Giam¹

¹Department of Microbiology and Immunology, Uniformed Services University of the Health Sciences; ²Department of Biological Chemistry UC Davis School of Medicine UC Davis Cancer Center

The regulatory circuit for Kaposi’s sarcoma-associated herpesvirus/human herpes virus 8 (KSHV/HHV-8) gene expression bears resemblance to that of Epstein-Barr virus (EBV), but with interesting differences. Based on protein sequence similarities and synteny to their EBV counterparts, two KSHV/HHV-8 viral regulatory factors, HHV-8 Rta and K-bZIP, encoded by ORF 50 and ORF K8, respectively, have been identified. Rta is an immediate early transcriptional activator that activates lytic viral replication and mediates viral re-activation from latency while ORF K8 is an early gene activated by Rta. Extensive splicing of ORF K8 mRNA leads to the production of K-bZIP — a protein of the basic domain-leucine zipper (bZIP) family. The role of K-bZIP in viral replication, however, remains unresolved. Here, we report that K-bZIP is a nuclear protein that binds Rta directly both in vivo and in vitro, and represses the Rta-mediated transactivation of the K-bZIP promoter. We further demonstrate that the leucine zipper domain of K-bZIP is required for Rta binding and a K-bZIP mutant lacking the leucine zipper no longer represses Rta activity. Finally, the K-bZIP-mediated repression of Rta transactivation cannot be restored by over-expression of the transcriptional co-activators p300 or p300-CBP associated factor, P/CAF. Our results suggest that K-bZIP is involved in a feedback circuit to turn off its own expression and possibly the expression of other early genes activated by Rta.
KSHV/HHV-8 TRANSCRIPTIONAL ACTIVATOR, RTA, IS AN OLIGOMERIC DNA-BINDING PROTEIN THAT INTERACTS WITH TANDEM ARRAYS OF PHASED A/T-TRINUCLEOTIDE MOTIFS

Wei Liao, Yong Tang, Yu-liang Kuo, Bao-Ying Liu, Chi-Jie Xu and Chou-Zen Giam

Department of Microbiology and Immunology, Uniformed Services University of the Health Sciences

Kaposi's sarcoma associated herpes virus (KSHV)/human herpes virus type 8 (HHV-8) encodes an immediate early transcriptional activator, Rta, which mediates viral re-activation from latency and lytic viral replication. Here we report the purification and characterizations of HHV-8 Rta, and its interaction with Rta-responsive DNA elements. The Rta response element (RtaRE) in the promoter of the KSHV/HHV-8 K8 open reading frame (ORF) was mapped to a 47-bp sequence. A comparison of the 47-bp sequence with other viral RtaREs revealed a pattern of multiple A/T-triplets spaced with a periodicity of 10 or 20 base pairs. Substitutions of the A/T-trinucleotides of K8 RtaRE with G/C bases greatly diminished Rta responsiveness. By contrast, base substitutions in an out-of-phase A/T-trinucleotide sequence had no effect. Importantly, multimers of (\(\text{A/T}_3\))\(_N_7\) and \(N_5\text{A/T}_3N_6\text{A/T}_4\) motifs support strong Rta response in a copy number-dependent manner. No specific sequence in the spacer regions could be discerned. Potent Rta response, however, was obtained with phased A/T-trinucleotides with 7-bp spacers of high G/C-content. Lengthening of the phased A/T motifs or lowering of the G/C-content of the spacers resulted in a reduction in Rta response. Finally, \(E.\ coli\)-derived Rta is an oligomer of 440 kDa in molecular size and binds RtaRE as an oligomer. These results support a model of Rta transactivation wherein the subunits of the Rta oligomer make multiple contacts with a tandem array of phased A/T-triplets in the configuration of (\(\text{A/T}_3\))\(_7\text{G/C}_7\) repeats.
KSHV VIRAL BCL-2 (ORF16) PROMOTER REGION IS ACTIVATED BY HYPOXIA
Isabelle Widmer, David Davis, Muzammel Haque, Zhi-Ming Zheng, Robert Yarchoan. HIV and AIDS Malignancy Branch, CCR, NCI, Bethesda, MD.

We previously found that hypoxia can induce lytic replication in KSHV infected PEL cell lines and that it can directly activate two KSHV promoters (RTA and Orf 34) (DA Davis et al, Blood: 97; 3244, Haque unpublished). Hypoxia leads to accumulation of hypoxia inducible factors (HIF) that bind to hypoxia response elements (HRE). Hypoxia can increase expression of both anti-apoptotic (huBcl-2, IAP 2) and pro-apoptotic (BNIP 3, NIX) factors in cells. We explored the effect of hypoxia on the expression of KSHV viral Bcl-2 (vBcl-2), an anti-apoptotic early lytic protein. The vBcl-2 promoter region from two PEL cell lines (JSC-1 and BC3) was cloned into luciferase gene reporter constructs. Both virus strains were utilized in order to analyze the influence of a variable length multiple repeat in the promoter region. Transient transfections were performed in Hep 3B cells and the responses to hypoxia (1% oxygen) and cobalt chloride (CoCl₂), which both increase HIF levels, were assessed. Either promoter upregulated luciferase expression 5-6x when exposed to hypoxia. CoCl₂ led to a 3x increase. Although deletion and mutational analyses did not reveal a classic functional HRE, co-transfection with either HIF-1 or HIF-2 alpha expression vectors led to a 4-7x increase in normoxia, and a 15x increase in hypoxia. Sequential deletions from either end of the promoter identified a 30bp region that was essential for induction. Deletions of any part of this sequence totally abrogated the hypoxia response. We show that vBcl-2, a human Bcl-2 homologue, is inducible by hypoxia. Both hypoxia and hypoxia induced viral replication can trigger apoptosis. HIF induction by hypoxia is immediate, and placing expression of a viral anti-apoptotic protein under cellular control in hypoxia would afford the virus an instant anti-apoptotic response. Expression of vBcl-2 in hypoxia could also contribute to the pathogenesis of PEL, which develops in a relatively hypoxic environment.
A COMPARISON OF THE KSHV ORF57/MTA AND TK PROMOTERS DEFINES TWO CLASSES OF RBP-JK ELEMENTS TRANSACTIVATED BY ORF50/RTA

Stephen J. Lynch, Diana Palmeri, Wei Bu, Kyla Driscoll, and David M. Lukac, Dept. of Microbiology and Molecular Genetics and International Center for Public Health; Univ. of Medicine and Dentistry of New Jersey; Newark, NJ 07101

We have previously demonstrated that the KSHV lytic switch protein, ORF50/Rta, functions as a ligand-independent inducer of RBP-Jk (Recombination Signal Binding Protein-Jk; aka. CBF-1, CSL), the target of the Notch proto-oncogene signal transduction pathway. RBP-Jk elements are found in the promoters of numerous KSHV genes, and we have demonstrated genetically that the interaction with RBP-Jk is required for ORF50/Rta-mediated activation of the promoters of the ORF57/Mta and SSB (Single stranded DNA binding protein) genes. The heterogeneity of promoter elements responsive to ORF50/Rta (50REs) observed by us and others suggests however, diverse and complex mechanisms for promoter association by Rta that regulate progression through the viral lytic cycle.

Comparison of the 50REs from the ORF57/Mta, TK, and K8/K-bZIP/RAP genes reveals a bi-partite structure in the ORF57 promoter, consisting of two half elements that are independently conserved in the other two promoters. We demonstrate that the half element shared between the ORF57 and K8 promoters is a direct binding site for highly-purified ORF50/Rta protein, while the half element shared by the ORF57 and TK promoters is an RBP-Jk element. DNA binding and transactivation studies of the wild-type and mutant elements affirms that DNA binding by RBP-Jk is essential for ORF50/Rta-mediated activation of the ORF57 and TK promoters, but not the K8 promoter. Furthermore, DNA binding by ORF50/Rta to the ORF57 promoter, but not the TK promoter, strongly potentiates activation by RBP-Jk, suggesting a novel mechanism for regulation of RBP-Jk activity, and defines two classes of RBP-Jk elements in KSHV promoters.
T CELL MEDIATED IMMUNE RESPONSE TO KSHV ORF 65 AND ORF73 IN INDIVIDUALS WITH AND WITHOUT KS

Tonia Woodberry¹, Leah Henry¹, Paula O’Connor¹, Jennifer Davis¹, Dennis Osmond², Jeff Martin², David Scadden¹ and Christian Brander¹

¹Massachusetts General Hospital and Harvard Medical School, Boston and ²Department of Epidemiology and Biostatistics, University of California, San Francisco.

Cellular immune responses elicited by KSHV (HHV8) infection remain poorly defined. From published data and by inference from other viral pathogens, potential immunogenic regions of KSHV include ORF 65 (Capsid protein) and ORF 73 (LANA). In this study T cell responses directed against each of these two regions were analyzed by ELISpot, using overlapping peptide pools spanning ORF65 and ORF73 amino acid sequences. Immune responses in peripheral blood mononuclear cells from 90 HIV positive and 45 HIV negative individuals were evaluated. In addition, 20 of the HIV positive individuals were tested sequentially for cellular responses to either ORF 65 or ORF 73 peptide pools over a period of 2-6 months and at different stages of KS. The results indicate that CTL responses are more frequently detectable in HIV+ KSHV+ individuals when compared to HIV-ve, KSHV+ individuals. However, overall weak cellular immune responses were detected from HIV positive subjects with current KS suggesting that specific CTL activity may be lost in late stage disease. To address this, ongoing KSHV viral load analyses will be correlated to the breadth and magnitude of the cellular immune response.
51
ACTIVATION OF NUCLEAR FACTOR OF ACTIVATED T-CELLS (NFAT) BY HHV-8 ENCODED CHEMOKINE RECEPTOR HOMOLOGUE ORF74: EVIDENCE FOR COLLABORATION WITH HIV-1 TAT IN KS PATHOGENESIS

Shibani Pati1, James S. Foulke, Jr.1, Oxana Barabitskaya1, Jynho Kim2, B.C. Nair3, David Hone1, Jennifer Smart2, Ricardo A. Feldman2 and Marvin Reitz1,2.

1Institute of Human Virology, University of Maryland Biotechnology Institute, Baltimore, MD 21201, 2Department of Microbiology and Immunology, University of Maryland, Baltimore, MD 21201, 3Advanced BioScience Laboratory Inc. 5510 Nicholson Lane Kensington, MD 20895.

Infection with human herpesvirus 8 (HHV-8) is necessary but generally insufficient to cause Kaposi’s sarcoma (KS). Co-infection with HIV-1 in the absence of antiretroviral suppressive therapy increases the risk for KS by many orders of magnitude. HHV-8 encodes a chemokine receptor homologue, ORF74, which has been implicated in KS pathogenesis. Expression of ORF74 constitutively activates several signaling pathways, including NF-κB, and induces the expression of various pro-inflammatory and angiogenic factors, consistent with the inflammatory hyperproliferative nature of KS lesions. We show that ORF74 also constitutively activates nuclear factor of activated T-cells (NFAT), which also regulates the expression of inflammatory cytokines and related factors. NFAT activation by ORF74 depends on signaling through the PI3-K/Akt/GSK-3 pathway and results in expression of NFAT dependent cell surface molecules, pro-inflammatory cytokines, and pro-angiogenic factors. NFAT activation is greatly increased by the HIV-1 Tat protein, although Tat alone has little effect. Our data further support the idea that ORF74 contributes to KS pathogenesis by a paracrine mechanism and provide the first evidence of a collaborative role for HIV-1 Tat and HHV-8 ORF74 in KS pathogenesis. These data may help explain the enhanced prevalence of KS in individuals dually infected with HIV-1 and HHV-8.
DEVELOPMENT OF A HIGH-THROUGHPUT SCREENING ASSAY FOR IDENTIFICATION OF NOVEL INHIBITORS OF KSHV POLYMERASE AND PROCESSIVITY FACTOR

Dorjbal Dorjsuren¹, Angela Brennan¹, Xulin Chen², Weimin Zhu¹, Paula Roberts¹, Robert Shoemaker³, Robert Ricciardi², Shizuko Sei¹. ¹Laboratory of Antiviral Drug Mechanisms, SAIC-Frederick, ²Departments of Microbiology, and Biochemistry & Biophysics, University of Pennsylvania, ³Screening Technologies Branch, NCI-Frederick.

The DNA polymerase (POL) and processivity factor (PF) of Kaposi’s sarcoma-associated herpesvirus (KSHV) are excellent molecular targets for anti-KSHV intervention because of their absolute co-requisite in viral DNA synthesis and high inter-molecular specificity. To facilitate discovery of novel inhibitors of KSHV POL/PF, we developed a microplate-based DNA synthesis inhibition assay, which could be applied to high-throughput screening (HTS) of large chemical libraries. Feasibility for screening was first examined by using in vitro translated POL/PF. Reproducibility of the optimized POL/PF inhibition assay was validated by replicate testing of the NCI Training Set, a collection of 230 well-characterized compounds, with r² of 0.903 observed at 20 µM test concentration. The established assay was further applied to screening of the NCI Diversity Set, which comprises approximately 2000 synthetic compounds, generating 28 hits with ≥50% inhibition. Concomitant with the assay optimization effort, we also generated recombinant baculovirus vectors to produce functionally active KSHV POL and PF (rPOL and rPF) in the large quantities required for HTS. The specificity of the rPOL/rPF-based assay was comparable to the in vitro translated protein-based assay. The inhibitory activities of 25 of 26 hit compounds examined were confirmed by the rPOL/rPF-based assay with the 50% inhibitory concentrations ranging from 0.12 ± 0.07 (mean ± SD) to 10.83 ± 4.19 µM. Automation of the KSHV rPOL/rPF assay is currently being pursued to promote future HTS campaigns. (Supported in part by NCI Contract No. NO1-CO-12400)
CYCLOOXYGENASE-2 REGULATES vGPCR ANGIOGENESIS AND TUMORIGENESIS

Agata Mutlu, Pilar Eroles, Chiara Chiozzini and Enrique A. Mesri. Laboratory of Viral Oncogenesis. Division of Hematology-Oncology. Department of Medicine, Weill Medical College of Cornell University, New York. eamesri@med.cornell.edu

KSHV G-protein-coupled receptor (vGPCR) is a viral oncogene that could exploit cell-signaling pathways to induce cell transformation and angiogenesis in KSHV-mediated Kaposi’s Sarcoma (KS) pathogenesis. Since cyclooxygenase-2 (Cox-2) have been largely implicated in carcinogenesis and in angiogenesis, the aim of this work was to investigate if vGPCR could regulate Cox-2 expression and how Cox-2 activity is involved in the angiogenesis activation by vGPCR. We found that vGPCR regulates Cox-2 activity and expression in NIH3T3 transformed cells. vGPCR-transformed cells produced seven times more PGE2 than the NIH3T3 controls. PGE2 synthesizing activity was completely inhibited by NS398, a specific Cox-2 inhibitor. Cox-2 protein levels measured by immunoblotting and cDNA levels measured by RT-PCR were increased in vGPCR-transformed cells. To investigate the involvement of Cox-2 on the activation of angiogenesis by vGPCR, we carried out an in vivo assay in which we quantified the formation of micro neo-vasculature. We found that nude mice inoculated with vGPCR-transformed cells showed significantly more and intricated neo-vasculature than NIH3T3 controls, the treatment with NS398 before inoculation completely abolished the increment observed in vGPCR expressing cells indicating that Cox-2 activity was necessary for angiogenesis. To analyze the impact of Cox-2 on vGPCR-mediated tumorigenesis we investigated the effect of treatment with Celecoxib, a FDA approved Cox-2 inhibitor, on the growth of vGPCR-transformed cell tumors in nude mice. We found that the treatment of mice with Celecoxib produced a consistent retardation in tumor occurrence and a significative decrease in tumor growth compared to control animals. This result indicates that Cox-2 activity contributes to the growth of tumor induced by vGPCR in mice. The finding that Cox-2 inhibition decreases vGPCR-transformed cells tumorigenicity and in vivo angiogenicity points this enzyme as possible target for KS therapy.
HUMAN HERPESVIRUS-8 (HHV-8) K1-ASSOCIATED NUCLEAR FACTOR -kB (NF-kB) AND VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) ACTIVATION: ROLE IN LYMPHOMAGENESIS IN K1 TRANSGENIC MICE.

Om Prakash¹, O. Rama Swamy¹, Zhen-Ya Tang¹, Xiaochang Peng¹, Roy Coleman¹, and Felipe Samaniego². ¹Ochsner Clinic Foundation, New Orleans, LA, and ²The University of Texas M.D. Anderson Cancer Center, Houston, TX.

The K1 gene of HHV-8 encodes a transmembrane signaling protein that elicits cellular activation events. We have previously reported that NF-kB, and Lyn, a Src proto-oncogene family protein-tyrosine kinase (PTK), were substantially activated in the B lymphocytes and B-cell lymphomas of K1 transgenic mice. Since VEGF has been implicated in HHV-8-associated disease pathogenesis, we investigated its potential role in K1-induced lymphomagenesis. We generated BALB/c nu/nu mice carrying K1 lymphoma sub-transplants by subcutaneous injection of a single tumor fragment (2 x 2 mm) in each mouse. The tumor fragments grew into tumors of ~2.5 cm diameter in about three weeks. Tumor-bearing mice showed a 2- to 3-fold increase in serum VEGF levels. Intraperitoneal administration of neutralizing anti-mouse VEGF (15mg/week, for three weeks) in the tumor-bearing mice resulted in a 45% to 50% decrease in the tumor growth. A cell line (KVL-1) derived from a lymphoma that constitutively expressed K1 and activated Lyn, produced high levels of VEGF (~200 pg/ml) compared with undetectable levels in the control culture medium. VEGF production markedly decreased when cells were treated with a Src PTK inhibitor PP2 (Calbiochem). Transfection of K1 in human B cells also induced expression of VEGF, and enhanced Lyn kinase catalytic activity. Treatment of these K-1 transfected cells with PP2 resulted in marked decreases in the NF- kB promoter and Lyn kinase activities, and lowered VEGF levels. Taken together, our results suggested that K1 might be involved in the activation of NF-kB and VEGF signaling pathways that lead to lymphomagenesis in K1 transgenic mice.
LYMPHATIC DYSFUNCTION IN KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS (KSHV) K-CYCLIN TRANSGENIC MICE

Makoto Sugaya¹, Aparche Yang¹, Mark Bryant², Mihaela Skobe³, and Andrew Blauvelt¹. ¹Dermatology Branch, NCI, ²Office of the Director, NIH, and ³Mount Sinai School of Medicine.

Lymphatic endothelial cells in adults express vascular endothelial growth factor receptor (VEGFR)-3. In addition, KSHV-infected tumor spindle cells within KS lesions express VEGFR-3, supporting the hypothesis that KS is derived from lymphatic endothelium. To further understand KS pathogenesis, we generated transgenic (Tg) mice that express the latent-cycle KSHV gene k-cyclin within lymphatic endothelial cells under the control of the murine VEGFR-3 promoter. When assessed quantitatively by real-time RT-PCR or by Western blotting, k-cyclin expression correlated with VEGFR-3 expression within a variety of tissues, and was highest in lungs. Clinically, in the Tg line with the highest level of k-cyclin mRNA expression, almost all heterozygous mice died prematurely within 6 months of age. Prior to death, CT scans of the lungs demonstrated progressive accumulation of fluid within pleural cavities that significantly compromised air spaces. Necropsies revealed 1) 0.25-1.5 ml of chylous fluid within pleural cavities; 2) diffuse atelectasis and severe congestion by routine histology; and 3) large dilated lymphatics by VEGFR-3 and LYVE-1 immunostaining. In addition, punctate petechiae were present on ears of Tg mice and histologically showed extravasated erythrocytes. Lastly, k-cyclin Tg mice demonstrated marked functional defects in lymphatic drainage following intradermal injection of either Evans Blue dye or FITC-conjugated dextran. In summary, lymphatic dysfunction with premature death secondary to respiratory insufficiency was observed in KSHV k-cyclin Tg mice. These mice provide a small animal model for assessing the in vivo function of KSHV genes and should thus prove useful in further understanding the pathogenesis of KS and other diseases associated with KSHV infection.
AWARENESS OF KSHV AMONG HOMOSEXUAL MEN
Alison Graves Jones, Dennis H Osmond, Lance M Pollack, Joseph A Catania & Jeffrey N Martin. Univ. of California, San Francisco

Background: Despite burgeoning scientific knowledge of Kaposi’s sarcoma-associated herpesvirus (KSHV) in the 9 years since its discovery and the presence of federal guidelines on preventing its transmission, little is known about what information has been translated to the community, particularly to homosexual men, the group at greatest risk for infection.

Methods: The California Health Interview Survey was a random digit-dial survey of 50,000 households throughout California conducted in 2001. Men who self-identified as homosexual, representing an estimated 398,668 homosexual men in California, were re-contacted in 2002 for a study of HIV-related sexual behavior. Participants were asked if they had heard of Kaposi’s sarcoma (KS) and, if yes, whether they knew the cause of KS.

Results: Of 402 homosexual men interviewed, most (82%) had heard of KS. Awareness of KSHV, however, was very uncommon:

<table>
<thead>
<tr>
<th>Reported Cause of KS (N=402)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not heard of KS/don’t know cause</td>
<td>48.0%</td>
</tr>
<tr>
<td>HIV/AIDS per se</td>
<td>41.8%</td>
</tr>
<tr>
<td>A “virus”, other than HIV</td>
<td>4.7%</td>
</tr>
<tr>
<td>A “herpesvirus”</td>
<td>0.8%</td>
</tr>
<tr>
<td>KSHV or HHV-8</td>
<td>4.2%</td>
</tr>
</tbody>
</table>

In an adjusted logistic regression model, HIV seropositivity (OR = 4.0, p<0.001) and postgraduate education (OR = 5.7, p<0.001) were associated with knowledge of KSHV (or a virus apart from HIV); there was no evidence of an association with age, race, interaction with the health care system, degree of sexual activity, or urban residence.

Conclusions: Awareness of KSHV is very low overall among homosexual men and only somewhat higher, but still unacceptably low, among HIV-infected men. Despite confusion over the route of transmission, which complicates the public health message, efforts are needed to increase general awareness of KSHV as an STD. The initial focus should be on HIV-infected men, the group at greatest risk for end-organ manifestations of KSHV infection.
ANALYSIS OF EARLY CHILDHOOD HUMAN HERPESVIRUS-8 INFECTION IN A ZAMBIA MOTHER/INFANT COHORT USING MULTIPLE SEROLOGICAL ASSAYS

Saul Phiri1,3, Tendai J. M’soka1,3, Geoffrey Kwenda1,2, Brad P. Brayfield1, Darius Simbeye3, Jubra Muyanga3, Chipeco Kankasa3, Gunapati J. Bhat2, John West1, Charles Mitchell4 and Charles Wood1

1Nebraska Center for Virology, University of Nebraska School Of Biological Sciences, Lincoln, USA; 2University of Zambia School of Medicine, Lusaka, Zambia; 3University Teaching Hospital, Lusaka, Zambia; 4University of Miami School of Medicine, Miami, USA

Background: Human Herpesvirus 8 (HHV-8) infection is endemic and associated with high incidence of Kaposi’s sarcoma (KS), limited serological studies have indicated that the presence of HHV-8 antibodies develop after birth and subsequently increase with age. Methods: We have used latent and lytic immunofluorescence assay (IFA) to conduct a longitudinal study at the University Teaching Hospital in Lusaka, Zambia with infants followed from birth. We have found a 15% infection rate using IFA but only 5% were confirmed by a commercial ELISA. To further resolve the differences between the assays, we developed several IFA assays based on a baculovirus expression vector system with either a lytic (orf 65 or K8.1A) or a latent (orf 73) gene using infected and uninfected insect cells. Results: We found that at one-year follow-up of a 485-mother/infant cohort the majority of the IFA results can be confirmed by the recombinant baculovirus assays. The HHV-8 seroconversion rate in the mothers was 15% whilst in he infants it was 18%. The 24 months samples are currently being analyzed. Conclusion: This data shows that in Zambian children HHV-8 infection appears to occur early in life and that HHV-8 infection amongst Zambian women and their children is quite prevalent, and multiple serological assays are important to confirm the results.
HHV8 (KSHV) TRANSMISSION IN AFRICA
Robert J. Biggar RJ1, S Mbulaiteye1, D Whitby2 and colleagues3.
1 Viral Epidemiology, NCI, MD; 2 SAIC, MD; 3 Nigeria/Tanzania.

Background In Africa, HHV8 prevalence is high, and transmission appears to be common in children. Routes of transmission have been unclear. We examined correlates of infection in different populations of East and West Africa.

Methods We studied HHV-8 infection in 2155 Nigerian adults with diverse sexual lifestyles. In addition, we did a cross-sectional family study (798 subjects) in rural Tanzania in a population without HIV infection. Infection status was determined by EIA-detected antibody to K8.1, a lytic antigen (6-8% indeterminate).

Results In Lagos, Nigeria, we found a low prevalence in the referent background population (22% of men and 14% of women). However, female commercial sex workers (31%) and both female (20%) and male (35%) attendees of clinics for sexually transmitted diseases had a higher prevalence than the referent population. Furthermore, within each group, persons who had laboratory evidence of a STD (chlamydia, HIV, HTLV, syphilis) were significantly more likely to be HHV8-infected.

In Tanzania, by age 3-4 years old, 58% of Tanzanian children were infected. Infection in childhood was associated with maternal HHV8 status (OR: 7.4; 95% CI 3.2-16.8) and less strongly with the infection status of other household members, including older siblings. In adults (≥18 years old), prevalence was high (88% in men; 79% in women). Even after adjusting for age, women with seropositive husbands were 6.9-fold more likely to also be seropositive than if their husbands were seronegative, indicating additional transmission occurs, probably via sexual intercourse.

Conclusions These studies show frequent transmission to children in high prevalence area, with some additional sexual transmission to those not infected at the age of initiating sexual activity. The mode of transmission to children may be salivary, but this does not explain transmission among adults.
59
MULTIPLE HUMAN HERPESVIRUS 8 INFECTION
Beyari MM\textsuperscript{1,2}, Hodgson TA\textsuperscript{1}, Cook RD\textsuperscript{1,2}, Kondowe W\textsuperscript{3}, Molyneux EM\textsuperscript{3}, Scully CM\textsuperscript{1}, SR Porter\textsuperscript{1}, Teo CG\textsuperscript{2}. 1Eastman Institute for Oral Health Care Sciences, University College London, UK;\textsuperscript{2} Central Public Health Laboratory, Public Health Laboratory Service, London, UK;\textsuperscript{3} University of Malawi, Blantyre, Malawi.

People living in HHV-8-hyperendemic regions may be repeatedly exposed to HHV-8. We investigated, in Malawian patients with KS and their relatives, nucleotide sequence variation in HHV-8 sub-genomic DNA amplified by PCR from mouth rinses, throat gargles, palatal scrapes and blood. Twenty-four people were identified who carried amplifiable HHV-8 DNA in >1 sample; 9 were HIV-1-seropositive, 21 anti-HHV-8-seropositive, and 7 exhibited KS. Sequence variation was sought in DNA segments derived from HHV-8 open reading frames 73, 26 and K1 using restriction fragment-length polymorphism analysis, nucleotide sequencing, PCR cloning and denaturing gel gradient electrophoresis. For 3 KS patients, genotypic differences were found between sequences from oral and blood samples. For 2 other KS patients and 9 people without KS, intra-person genotypic and sub-genotypic differences were found in oral samples; intra-sample carriage of distinct HHV-8 strains could be found in 2 KS patients and 4 non-KS individuals. Our findings imply HHV-8 superinfection and bear impact on future epidemiological studies, and on the design of vaccines against HHV-8.
DETERMINANTS OF HHV8 VIREMIA WITHOUT HIV/AIDS


Individuals with detectable human herpesvirus 8 (HHV8) DNA in peripheral blood mononuclear cells (PBMC) are at heightened risk for developing Kaposi’s sarcoma (KS). Using data and specimens from HIV-negative, KS-free volunteers in Sicily, Rome and Naples, we assessed determinants for the presence of PBMC HHV8 DNA among individuals manifesting antibodies against HHV8 LNA by IFA. We used TaqMan real-time PCR to quantify HHV8-K6 copies/10^6 cells in DNA from cryopreserved PBMC standardized to the human single copy ERV3 gene. Blood counts were quantified by automated hematology analyzer and T-lymphocyte subpopulations by flow cytometry on fresh whole blood. Data were analyzed by logistic regression. Among 158 KS-free adults with HHV8 latent infection, 16.5% demonstrated detectable levels of HHV8 DNA in PBMC (median=53; range=13-2128 copies/10^6 cells). No difference was seen by gender (18.8% female), age (mean = 75.6 years with viremia) or geographic region (21.2% from Rome/Naples). Compared to controls, HHV8 DNA was detected more often in persons with crowding in the childhood home (OR=3.66 95% CI 1.47-9.14), more than 2 younger siblings (OR=2.57 95% CI 1.01-6.51), a current history of cardiovascular (OR=3.58 95% CI 1.31-9.74) or renal disease (OR=3.05 95% CI 1.16-8.01), thrombocytopenia (<159 K/µL; OR=28.22 95% CI 4.96-160.55) and among women, more than 1 total lifetime sex partner (OR=29.46 95% CI 2.24-388.34). In support of these findings, higher levels of HHV8 DNA load were modestly associated with renal disease and thrombocytopenia (p 0.15) and significantly associated with the other covariates (p 0.05). The detection of HHV8 DNA was highly correlated with several factors including exposures from childhood than can ultimately confer risk for viremia as an adult. These findings are the first to be reported with respect to risk factors for HHV8 viremia.
CONTROL OF AIDS-ASSOCIATED KAPOSI’S SARCOMA THROUGH ANGIOGENESIS INHIBITION
A. Albini¹, M. Morini², U. Pfeffer¹, S. Ferrini³, K. Mölling⁴, R. Benelli, N. Ferrari ¹, and D.M. Noonan², ¹Molecular Oncology, ²Tumor Progression and ³Immunofarmacology; Instituto Nazionale per la Ricerca sul Cancro, Genova, Italy and the ⁴University of Zurich, Zurich, Switzerland

AIDS-Associated Kaposi's sarcoma (KS) is a highly angiogenic lesion with a high risk for recurrence on HAART failure in HHV8 infected individuals. Angiogenesis is necessary for both tumor growth and metastasis. We have examined several pre-clinical anti-angiogenesis approaches to the control of KS, including angioprevention agents and gene therapy.

The synthetic retinoid N-4-hydroxyphenyl-retinamide (fenretinide or 4HPR) is an effective chemopreventive agent whose molecular mechanisms are poorly understood. 4HPR inhibited the growth of established KS xenografts in nude mice. Histological analyses showed a significantly lower vessel density in the tumors of 4HPR treated animals, suggesting direct effects on tumor angiogenesis. 4HPR did not affect KS cell viability or growth in vitro, but inhibited growth, morpho-genesis, chemotaxis and invasion of endothelial cells in vitro and angiogenesis in vivo in the Matrigel sponge assay. 4HPR rapidly reduced the expression of vascular endothelial growth factor (VEGF) by KS cells and of the VEGF receptor, VEGFR2, on the surface of endothelial cells and decreased release of MMP-2. These data indicate that the chemopreventive drug 4HPR represses KS angiogenesis at several different levels, an angioprevention agent.

Gene therapy delivery of angiogenesis inhibitors is an ideal strategy for chronic and effective anti-angiogenic therapy. IL12 is a heterodimeric TH1 cytokine that possesses anti-angiogenic activities thought to be mediated through T and NK cell IFNα secretion and subsequent IP-10 induction. Naked DNA intra-muscular injection of an IL-12 expression vector gave significant elevation of serum IL-12. Injection of the IL-12 vector at least 2 days, and up to 20 days, before the matrigel sponge assay resulted in strong inhibition of angiogenesis in both C57/bl and nude mice. Injection of the IL-12 vector contemporarily with matrigel did not significantly inhibit angiogenesis. Angiogenesis inhibition was also observed in NK cell depleted C57/bl and nude mice as well as in IFNα knock-out mice, indicating that NK and T-cell initiated IFNα cascades were not involved in the angiogenesis inhibition observed in vivo. Finally, IL-12 vector DNA gene transfer significantly prevented the growth and vascularization of highly angiogenic KS-Imm tumors in vivo. These data suggest that a gene therapy approach can effectively inhibit KS tumor growth.
MORPHOLOGY AND MECHANISMS OF REGRESSION IN AIDS-RELATED KAPOSI'S SARCOMA (KS)
L Pantanowitz1, SR Tahan1, GS Pinkus2, BJ Dezube3. 1Depts of Pathology and Medicine (Hem/Onc Division), Beth Israel Deaconess Medical Center; and 2Dept of Pathology, Brigham and Women’s Hospital; Harvard Medical School, Boston, MA, USA

INTRODUCTION: KS can regress or undergo progression into a true sarcoma. Progression is related to human herpesvirus-8 (HHV8)-induced expression of latent nuclear antigen 1 (LNA-1), cyclin D1 and bcl-2. More recently, c-kit expression has been shown to play a role in KS tumorigenesis. Regression of AIDS-associated KS may occur spontaneously or following antiretroviral and/or KS-directed therapy. However, very little has been published on the pathologic findings and mechanisms of regression in KS. Therefore, the aim of this study was to characterize the histopathology of regressed KS lesions and determine the mechanisms associated with regression in vivo following therapy.

METHODS: Eight HIV-positive male patients (median age 41 years; range 32-48) with untreated or persistent (despite HAART and prior KS treatment) cutaneous KS that demonstrated regression of their KS (in accordance with the AIDS Clinical Trial Group criteria) following either paclitaxel or COL-3 (an investigational angiogenesis inhibitor) treatment were selected. Skin 4-mm punch biopsies of KS lesions (1 patch, 6 plaque and 1 nodular) obtained prior to and after therapy were compared. In addition, immunohistochemical stains for the endothelial marker CD31, LNA-1, cyclin D1, bcl-2 and c-kit (CD117) were performed.

RESULTS: KS in all patients underwent complete or partial histological regression following therapy. Regression clinically resulted in shrunken, brown-pigmented cutaneous macules. Median HIV-1 viral load pre-therapy was 4,310 and post-therapy 1,100 copies/ml. Median CD4+ cell count pre-therapy was 270 and post-therapy 380 cells/mm³. Completely regressed lesions showed an absence of spindle cells. Partial regression was characterized by the presence of some residual spindle cells. In all regressed lesions hemosiderin-laden macrophages and an increased perivascular reactive lymphocytic infiltrate were found. Regression was accompanied by a reduction in CD31, LNA-1 and cyclin D1 immunoreactivity, and no change in bcl-2 or c-kit staining.

DISCUSSION: AIDS-associated KS underwent histological proven regression following paclitaxel and COL-3 therapy. Spindle cell regression and diminished CD31 expression were accompanied by an improvement in host immunity (infiltrating lymphocytes and increased CD4+ counts) and concomitant decline in HIV-1 viral load. Regression was also attended by a reduction in LNA-1 and cyclin D1, and no change in bcl-2 expression. KS spindle cells in all pre-treatment and partially regressed lesions demonstrated strong c-kit positivity, identifying an additional potential target for future pharmacological intervention.
PHASE I-II TRIAL OF BMS-275291 IN PATIENTS WITH HIV-RELATED KAPOSÍ’S SARCOMA: A MULTICENTER TRIAL OF THE AIDS MALIGNANCY CONSORTIUM

Von Roenn JH for the AIDS Malignancy Consortium

BMS-275291 is an orally bioavailable rationally designed peptidomimetic matrix metalloproteinase inhibitor (MMPI) with a chemically novel mercaptoacetyl group as the zinc-binding group. The compound was designed to potently and selectively inhibit MMP-2 while sparing membrane protein metalloproteinases that regulate the cell surface shedding of TNF-α and TNF-α RII. It was hoped that this would limit the arthritis/arthralgia seen with hydroxamate-based MMPIs. Matrix metalloproteinases promote tumor-mediated angiogenesis, a key feature of Kaposi’s sarcoma (KS). KS-derived cell lines release MMP-9 constitutively into culture medium, and tissue inhibitors of MMP-2 inhibit KS-like lesions induced by KS cell-free supernatants in mice. These data suggest that MMPIs are potential therapeutic agents for KS.

The objective of this trial was to evaluate the toxicity, tolerability, and efficacy of BMS-275291 in patients with AIDS-related KS and to explore the relationship between tumor response and biologic endpoints.

Sixteen patients were enrolled on this study, 15 of whom received drug. 8 patients were assigned sequentially to level 1 (1200 mg once a day) and 7 to level 2 (600 mg bid), but one level 1 patient inadvertently received only 600 mg/d (level 0). Eligible patients had biopsy-proven KS and documented HIV infection. Four weeks of continuous therapy constituted one cycle. Mean age was 40.9 years (range 32 to 60), 100% were male, and median CD4 count was 263 cells/μl (range 15 to 707).

Grade 3 neutropenia and liver function abnormality were each observed in 1 of 7 level 1 patients. Grade 3 granulocytopenia and liver function abnormality were observed in 2 of 7 and 1 of 7 level 2 patients, respectively. Three level 2 subjects discontinued treatment because of grade 3 toxicities: severe fatigue (1), severe arthralgias (1), and an allergic reaction (1). Mean and median treatment duration were 23.2 and 20 weeks, respectively. Cutaneous tumor biopsies pre- and post-treatment were obtained to assess the change in percent apoptotic cells. No firm conclusions could be drawn as to the effect of treatment on apoptosis.

Partial response of KS was observed in 1/1 patient at level 0 after one year of treatment; 0/7 on level 1, and 2/7 at level 2 within one month of treatment. Durations of responses were 1+, 7+ and 8+ months. Five out of 7 level 1 patients and 3/7 level 2 patients had stable disease.

CONCLUSIONS

For this patient population, 1,200 mg once a day was the MTD for BMS-275291, but no antitumor responses were seen at this dose level.
MICROARRAY EXPRESSION PROFILE IN HIGH-GRADE AND LOW-GRADE SQUAMOUS INTRAEPITHELIAL LESIONS

Bruce J. Dezube, George Tseng, Dana Fugelso, Harold Greenberg, Imad Nasser, L.J. Wei, Pamela A. Chatis. Beth Israel Deaconess Medical Center, Harvard Medical School; Harvard School of Public Health; and PerkinElmer Life Sciences; Boston, MA. AIDS Malignancy Consortium, National Cancer Institute.

PURPOSE: To compare RNA gene expression differences of high-grade (HSIL) and low-grade (LSIL) anal cancer patients by microarray expression profile. PATIENTS AND METHODS: Anal biopsy specimens were collected in RNA Later I and then transferred into Trizol reagent. Total RNA was extracted, quantified, reverse transcribed to cDNA, and labeled with haptenated nucleotides. Tagged DNA was then hybridized to human cDNA probes on the Miromax 4800 gene array. A total of 21 paired cDNA microarray slides were analyzed. Each pair consisted of cDNA isolated from two biopsy samples obtained from a single patient (5 normal anus paired with normal anus, 9 HSIL paired with normal anus, and 7 LSIL paired with normal anus). Permutation testing was used to select sets of genes that were statistically significant. Clustering was used to sort out clusters of genes that behave similarly. RESULTS AND DISCUSSION: By comparing HSIL to normal and LSIL to normal, we detected 9 common genes among the 27 LSIL and 47 HSIL up-regulated genes and 10 common genes among the 34 LSIL and 51 HSIL down-regulated. The expressions in these 19 genes were significantly different when HSIL and LSIL samples were compared to normal ones. By comparing HSIL to LSIL, we identified 9 up-regulated genes in HSIL and 9 down-regulated ones. Five clusters of genes with significant patterns were identified. Up-regulated HSIL genes: Notch3, Cystatin B, ACTB mRNA for mutant beta-actin, hMSH6, Lysosome-associated membrane glycoprotein, HMG-Y protein isoform mRNA (HMG), mRNA for lipocortin II, Vacuolar H+ ATPase proton channel subunit, Clone 23827 heat shock protein. Down-regulated HSIL genes: BTG1 mRNA, mRNA for seryl-tRNA synthetase, MAD-3 mRNA encoding IkB-like activity, Ribosomal protein L12, Global transcription activator homolog, Integrin-linked kinase (ILK), Translation initiation factor elF3 p40 s, Transducin-like protein, Wnt-13 mRNA. Differential gene expression between HSIL and LSIL lesions may point to biological pathways and/or molecular interactions, which can help elucidate the mechanism by which an intraepithelial lesion assumes a more aggressive/malignant phenotype.
Human papillomavirus (HPV) has been implicated in some cases of head and neck cancer (SCCHN). The tumorigenicity of the virus depends on whether its genome is integrated or episomal. Expression of the early open reading frame (ORF) E2 gene negatively regulates the levels of the viral oncogenes encoded by the E6 and E7 ORFs. A breakpoint within the E2 sequence allows the circular genome to integrate into the cellular genome, which results in the upregulation of E6 and E7. These viral oncoproteins promote tumorigenesis by disrupting the function of p53 and pRb. Integration may also result in insertion mutagenesis, thereby disrupting tumor suppressor genes or activating proto-oncogenes.

We have established a HPV16-positive SCCHN cell line (UPCI:SCC090) derived from the base of the tongue of a 44 year old male with a history of smoking. Karyotypic analysis revealed numerous numerical and structural chromosome abnormalities. HPV FISH revealed multiple copies of integrated HPV16 viral DNA in blocks throughout the genome. To assess whether these integration sites coincide with known viral integration sites and/or chromosomal fragile sites, we have mapped integration by FISH in combination with spectral karyotyping. Integration sites were identified on chromosomes 3p, 3q, 6p22, 6q, 9q22, 12p, 13q and t(1;8)(q; ?). To identify whether specific genes have been interrupted by the viral integration process, viral/human junctions are currently being mapped by restriction site polymerase chain reaction (RS-PCR). Preliminary results confirm one integration site at 9q22.32.
DETECTION OF PERIPHERAL T CELL RESPONSES TO HPV16 E7 PEPTIDES IN HUMANS BY THE CFC ASSAY.
Malcolm John¹, Joel Palefsky¹, C. Lorrie Epling¹, Elizabeth Sinclair¹, Barry M. Bredt¹, Joseph M. McCune¹,². ¹University of California, San Francisco; ²Gladstone Institute of Virology and Immunology.

Introduction: Human papilloma virus (HPV)-associated anal intraepithelial neoplasia (AIN) may increase significantly in HIV+ individuals. To better study the natural history of and immunologic responses to anogenital HPV infection, we developed a cytokine flow cytometry (CFC) assay to detect T cell responses to HPV.

Methods: Peripheral blood or peripheral blood mononuclear cells (PBMC) from HPV+ men were stimulated with antibodies against CD28/49d as well as 15mer peptides that spanned the HPV16 E7 sequence. Brefeldin A was added at 2hrs and cells were analyzed after 4 more hrs by 4-color flow cytometry. Antigen-reactive T cells co-expressed CD69 and intracellular interferon-γ; responses ≥ 0.05% (adjusted for background) were scored as positive.

Results: 4 of 20 HIV+ men with AIN responded in whole blood assays (CD8 0.14 - 0.60%; CD4 not positive); all 3 with follow-up tests had consistent responses. In 7 HIV- men with history of AIN, PBMC and longer CFC reactions were used. More robust responses persisting over 3 visits were seen in 2 men with only remote low-grade AIN (CD4 0.53 – 0.93% in one, 2.82 – 15.11% in another, larger responses seen with longer CFC reactions; CD8 0.08 – 0.52% but seen only with longer reactions). Findings were supported by responses to our peptides in a proliferative assay and in antigen-specific T cell expansion assays in-vitro.

Conclusions: To our knowledge, this is the first report of peripheral responses to HPV antigens detected in humans utilizing the CFC assay. We have identified 5 responders positive over ≥ 2 visits. The small number of responders may reflect the fact that HPV resides in the epithelium and systemic responses therefore expected to be small. Further work remains to establish the consistency of these early results with subsequent optimization and standardization of the assay.
SPLICING OF A CAP-PROXIMAL HUMAN PAPILLOMAVIRUS 16 E6E7 INTRON PROMOTES E7 EXPRESSION, BUT CAN BE RESTRAINED BY DISTANCE OF THE INTRON FROM ITS RNA 5' CAP

Zhi-Ming Zheng, Mingfang Tao, Sohrab Bodaghi and Wei Xiao
HIV and AIDS Malignancy Branch, Center for Cancer Research, NCI/NIH, Bethesda, MD, USA

Human papillomavirus 16 (HPV16) E6E7 pre-mRNA has an intron in the E6 coding region with one 5' splice site and two alternative 3' splice sites, producing E6*I and E6*II. The E6E7 mRNA with this intron unspliced expresses oncogenic E6. We found for the first time that the E6E7 pre-mRNA was spliced in vitro only when capped and cellular cap-binding factors were involved in the splicing. Cap-dependent splicing of the E6E7 pre-mRNA was extremely efficient in cervical cancer-derived cells, producing mostly E6*I, but inefficient in retrovirus expression vector pLXSN16E6E7-transfected cells, due to the large size (>1 kb) of its exon 1. When cloned in different positions of other mammalian expression vectors, the E6E7 pre-mRNA expressed with a cap-proximal intron in a distance of 180 nts from its 5' cap was spliced efficiently, but when the cap-proximal E6E7 intron was approximately 941 nts from its cap, it was spliced poorly. The same was true for splicing of human β-globin RNA. Further size-limiting studies revealed that an optimal in vivo splicing of the E6E7 pre-mRNA requires a cap-proximal intron in a distance less than 333 nts from its RNA 5' cap and that the splicing provides more E7 RNA templates for E7 expression. Data indicate that the distance between RNA 5' cap and cap-proximal intron is rate limiting for cap-dependent RNA splicing and protein expression.
AN IMPORTANT ACCESSORY ROLE FOR THE CERVICAL CANCER RISK FACTORS ENCODED BY HUMAN PAPILLOMAVIRUS E6 AND E7 ONCOGENES IN HIV-INDUCED AIDS. PR Manuri¹, EM Vela¹, PN Nehete¹, JK Sastry¹. ¹Dept of Veterinary Sciences, The Univ of Texas M.D. Anderson Cancer Center

Infection by the high-risk Human Papillomavirus type 16 (HPV-16) has been well established as a major risk factor for cervical neoplasia, and HPV-associated cervical cancer has also been recognized as a major AIDS-associated malignancy in HIV infected women. However, a clear role for either HIV in HPV-associated cervical neoplasia or HPV in the HIV-induced AIDS has not been established. We obtained evidence to show a clear involvement of the E6 and E7 oncoproteins encoded by the high-risk HPV-16 in enhancing the replication of HIV. In particular, we demonstrate that both E6 and E7 independently interact with tat, an HIV-encoded regulatory protein essential for HIV replication.

Transient co-transfection experiments using plasmids encoding the full-length HIV genome and either the E6 or E7 oncogenes of HPV-16 showed a 3 and a 2.2 fold increase in viral DNA levels, respectively as measured by the polymerase chain reaction (PCR). Further, the resulting virus from the culture supernatants, when tested on indicator HeLa CD4⁺ β-gal cells showed 2-4 fold increase in infection. These experiments clearly show that the E6 and E7 oncogenes enhance HIV-1 replication and virus production.

We hypothesized that the enhancing effects of the E6 and E7 oncogenes on HIV replication might be mediated through cooperation with tat, the potent transactivator of viral gene expression from the 5’ long terminal repeat (LTR) promoter located in the HIV-1 genome. Reporter plasmid assays with HIV-1 LTR linked to the chloramphenicol acetyl transferase (CAT) gene were carried out. Human pancreatic epithelial cells, Panc-1 were co-transfected with HIV-1 LTR-CAT and a plasmid encoding HIV-1 Tat (pSVtat72) in the presence or absence of either the E6 or E7 plasmids. We observed 2 and 3 fold increases in CAT activity in the presence of E6 and E7, respectively. Enhanced expression of HIV-1 LTR-CAT was also observed when the tat protein was supplied as supernatant from Cos-1 cells transfected with a tat-expression plasmid. Further, transient transfection experiments employing the human T cell line Jurkat or a derivative Jurkat-tat, with stable tat expression, yielded enhancement of CAT activity by 2.7 to 9 fold at various concentrations of E6 and 2.7 to 12 fold at various concentrations of E7. Together, these results provide compelling evidence for an active role by HPV16 E6 or E7 oncogenes in enhancing HIV-1 replication mediated through cooperation with the tat.

Thus, these findings suggest an association between HIV-1 tat and E6 or E7 at the molecular level which may be of relevance in the contest of rapid progression of AIDS in HPV infected patients. Further probing into these aspects might help in the understanding of this phenomenon.
4,4’-DIHYDROXYBENZOPHENONE-2,4-DINITROPHENYLHYDRAZONE (A-007) - A MODULATOR OF CD45+ T-LYMPHOCYTE IMPAIRED ANO-GENITAL EPITHELIUM. L.R. Morgan¹, C.L. Hooper¹, A. Rodgers¹, V.J. Culotta², J. H. Bellina³ and R. W. Kelly³. ¹DEKK-TEC, INC, New Orleans, LA, ²East Jefferson Hospital, Metairie, LA and ³Omega Institute, Metairie, LA.

A-007 is an aryl hydrazone that has immune modulating properties when applied topically, intravaginal or intra-anal, as a 0.25% gel to pts with cancer of anogenital origins. In other studies, A-007 has demonstrated >25 % objective responses when applied topically to patients with metastatic breast cancer, melanoma and lymphomas [NCI-EORTC, Abst. 477, 1998 and AACR, Abst. 4825, 2002]. In the current study, 10 patients with cancers of the cervix (7), anal (1) or vagina (2) were treated daily for 5-days with 2 g of a 0.25% A-007 gel. 8/10 patients were HPV+ and 0/10 HIV+. Pre- and post-A-007, tissue biopsies, complete blood counts, chemistries, urine analyses and plasma levels for A-007 were obtained. Biopsies of A-007 treated anogenital sites revealed increased infiltrations of CD3+/ CD4+/ CD8+/CD45+ T-lymphocytes after only 5-days of treatment with no local toxicity. To date - 6/CR, 1/PR and 3/NC have been noted. For the one anal cancer treated - a CR was observed. HPV titers in 6/8 patients decreased statistically. This agrees with intravaginal A-007 studies in monkeys and rabbits - no related acute contact dermatitis (ACD) and no infiltrates of neutrophils or eosinophils were noted. A-007 was not detected in any plasma samples, nor were changes noted in CBCs, chemistry profiles or urines for any pt during or after treatments. The most obvious consistent immunohistochemical change noted (for tissue and blood) was an up-regulation of CD45+ T-lymphocyte receptors. A-007 can exist as a tissue hapten capable of generating extra-nodal immune modulations via lymphatic networks. Interactions with CD45+ surface receptors and T-lymphocyte activation-cascades will be discussed. Future plans to use A-007 as an immune modulator and/or a co-modulator of HPV/HIV associated anogenital cancers will be discussed.
ANAL PAP AND ONCOGENIC HUMAN PAPILLOMAVIRUS (HPV) SHEDDING DO NOT PREDICT HISTOLOGIC HIGH-GRADE ANAL INTRAEPITHELIAL NEOPLASIA (AIN) IN MEN WHO HAVE SEX WITH MEN (MSM)

Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

Introduction. HPV-associated AIN is increased in men who have sex with men (MSM), especially in those with HIV coinfection. Clinical presentation, pathology results and anal HPV shedding were compared in HIV+ and HIV- MSM at our institution.

Methods. Charts of MSM referred to the Infectious Diseases Dysplasia Clinic between 4/99 and 1/03 were reviewed. History of anal condylomata (AC), paired cytology and biopsy results, and anal shedding of high-risk HPV types were compared between HIV+ and HIV- groups using Chi-square and Fisher’s exact tests.

Results. Of 168 MSM, 101 (60%) were HIV+ and 67 (40%) were HIV-. At the initial visit, more HIV+ vs. HIV- MSM reported a history of internal AC (39% vs. 13%) and/or external AC (64% vs. 36%) (p<0.05). 40% HIV+ and 67% HIV- MSM with no history of internal AC were found to have internal AC at colposcopy (p=0.02). 34% HIV+ and 54% HIV- MSM with low-grade Paps had high-grade AIN on paired histology. Anal shedding of oncogenic HPV occurred in 80% of both groups and did not correlate with grade of AIN on Pap or biopsy.

Conclusions. History of AC is more common in HIV+ MSM, though more HIV- MSM were newly diagnosed with internal AC. Both groups had a substantial incidence of low-grade Paps paired with high-grade histology results. Shedding of oncogenic HPV types was common and did not help to predict the grade of AIN on Pap or biopsy. Any grade of abnormal anal cytology suggests the possibility of high-grade histology, and HPV testing does not aid in indicating those at risk for high-grade AIN.
SIMULTANEOUS DETECTION OF HPV DNA AND MALIGNANT CELL PROLIFERATION MARKERS AS POTENTIAL TOOLS FOR CERVICAL CANCER DIAGNOSTICS AND SCREENING

Jay Ji, Hiro Nitta, Lidija Dragovich, Kimberly Christensen and Tom Grogan
Ventana Medical System Inc., Tucson, AZ

The high prevalence of HPV in cervical carcinoma worldwide has established HPV as a central etiologic factor in cervical oncogenesis, with about 400,000 cancer cases diagnosed annually. However, in vitro cell-free based HPV DNA assay as a sole diagnostic marker has limited utility due to the high HPV infection frequency for woman with either normal or atypical squamous cells of undetermined significance (ASCUS) while advanced carcinoma has very low or undetectable viral titers. Using cytology based in situ hybridization, Ventana has fully automated HPV DNA test for pathology laboratories to detect both high and low risk of HPV from cervical specimens. We now evaluate and integrate several cell proliferation markers in conjunction with HPV detection. An automated assay for simultaneous detection of HPV DNA in situ and malignant cell proliferation markers P16 and Ki67 should enhance significantly overall management and triage of ASCUS, cervical cancer diagnostic and treatment monitoring as well as primary population screening.
THE OPTIMAL ANAL CELL COLLECTION TECHNIQUE FOR SCREENING ANAL INTRAEPITHELIAL NEOPLASIA IN HOMOSEXUAL MEN. J. Anderson 1,2, C. Vajdic 1, R. Hillman 3, G. Medley 4, A. Grulich 1. 1National Centre in HIV Epidemiology and Clin Res, Univ of New South Wales, 2Carlton Clinic, 3Sexually Transmitted Infections Res Ctr, Westmead Hosp and 4Victorian Cytology Svc.

Introduction: Detection of intraepithelial neoplasia of the cervix by cytology is used as the basis for screening to prevent cancer of the cervix. An increasing awareness of the high rates of anal intraepithelial neoplasia (AIN) and anal cancer in homosexual men has led to a recommendation for anal screening of this population. The anal sampling method must make available an adequate number of cells for cytology and ancillary studies, in particular tests for HPV infection, yet there are no published comparisons of anal smear collection techniques.

Methods: Using a paired, random sequence design, we compared two methods of smear collection using a Dacron swab, one ‘blind’ and the other proctoscope-aided, in 200 homosexual men. Two trained clinicians performed the smears in a randomised order because of the unknown extent of any sequence effects. The ThinPrep procedure was used to prepare the cytologic specimens. High-resolution anoscopy and biopsy were performed on men with cytologically diagnosed high-grade AIN. A single, trained pathologist performed the cytopathology and pathology without knowledge of the smear method or results of any other tests. The two methods were compared with respect to the cytological classification of the specimen, the presence of rectal columnar, squamous and metaplastic cells, the presence of contamination, the specimen adequacy rate, patient comfort and acceptability, and the volume of fluid remaining after the ThinPrep procedure. The extent of any order effect was examined using Fisher’s Exact Chi-square test, and paired samples were analysed using McNemar’s Test.

Results: By 14th February 2003, results were available for 94 men (63 HIV-positive and 30 HIV-negative), in whom the prevalence of low and high-grade anal neoplasia was high (31% and 20% respectively). There were no statistically significant differences between the 1st and 2nd-performed smears. Compared to proctoscope-aided smears, blind smears were significantly more likely to be rated as adequate for cytological assessment (p=0.01). The blind and proctoscope-aided smear samples were similar for all other cytological measures, and there was no difference in patient comfort or acceptance between the two methods. The positive predictive value for high-grade AIN was 78% for blind smears and 83% for proctoscope-aided smears.

Conclusions: Preliminary findings suggest that direct visualization of the anal transformation zone is not necessary for accurate anal cell sampling.
GENITAL DYSPLASIA IN HIV INFECTED WOMEN

Gregory Taylor MD\textsuperscript{1,2}, Tracy Wolff MD\textsuperscript{1}, Niharika Khanna MD\textsuperscript{1}, Priscilla Furth MD, \textsuperscript{3} Patricia Langenberg PhD\textsuperscript{4}, \textsuperscript{1}University of Maryland, \textsuperscript{2} Institute of Human Virology, \textsuperscript{3} Georgetown University, \textsuperscript{4} University of Maryland Department of Epidemiology

Background: Women infected with HIV are at a greatly increased risk for the development of dysplastic genital lesions. Historically markers of immuno-suppression such as a decline in CD4+ count were predictive of the development of dysplasia. Recent advances in antiretroviral medical therapy can now restore a once depressed CD4+ cell count, while simultaneously suppressing HIV replication. In this new era additional predictive markers of genital dysplasia are needed for management of HIV positive patients.

Methods: An observational consecutive sample of 200 HIV infected women from an Urban University clinic was analyzed, in which measurements of histopathology, CD4+ count, CD4+ nadir, HIV viral load, HPV, and usage of HAART were correlated with genital dysplasia.

Results: There was detected an observable protective effect against any genital dysplasia from having HAART prescribed (Relative Risk = 0.77, 95\% CI 0.56, 1.06) and compliance with HAART (Relative Risk=0.61, 95\% CI=36, 1.02). High risk HPV DNA was a positive predictor of dysplasia p=0.0003. The most significant finding of importance was the correlation of a lower CD4+ count nadir being strongly associated with genital dysplasia (p=0.0003).

Conclusion: Greater immuno-suppression as measured by the nadir of a patient’s CD4+ count is the strongest predictor of genital dysplasia in HIV infected women. Future studies to better define this phenomenon are warranted.
THE ASSOCIATION OF ANTIRETROVIRAL THERAPY (ART) WITH ANAL SQUAMOUS INTRAEPITHELIAL LESIONS (SIL) AMONG HIV+ MEN

Timothy Wilkin¹, Steve Palmer², Karen Brudney², Mary Ann Chiasson³, Thomas Wright TC²
¹Weill Medical College of Cornell University, ²Columbia University, ³Medical Health Research and Administration, New York, NY

Background: Previous studies of HIV+ men have demonstrated a high rate of anal SIL- a precursor to anal carcinoma, but included predominantly white men who have sex with men and men not on effective ART. We examined the prevalence of anal SIL and high-risk HPV in a group of HIV+ men including men of color, men with and without a history of receptive anal intercourse (RAI), and those on and off effective ART. Methods: Cross-sectional study in an urban Infectious Diseases clinic. Ninety-two participants (53% Latino and 36% African American; 40% without a history of RAI) were evaluated with a questionnaire, Digene HC II HPV DNA assay and HPV DNA PCR for high-risk types of HPV, conventional and liquid-based cytology, and anal colposcopy with biopsy of visible lesions. Predictors of anal HPV, abnormal cytology, and anal SIL on biopsy were determined by chi square test, and logistic regression. Results: 61% had high-risk HPV and this was related to a history of RAI (78% vs. 33%, p=.008). 47% had abnormal liquid–based cytology and 40% had anal SIL on histology, and both were associated with a history of RAI, lack of current ART use, and lower nadir CD4 count on multivariate analysis. The association of ART and nadir CD4 with SIL remained significant after controlling for the presence of high risk HPV. Conclusions: Although anal high-risk HPV infections and SIL in HIV+ men are associated with a history of RAI, both conditions are commonly identified in HIV+ men who deny RAI. Using multivariate analysis both nadir CD4 and no current ART use are associated with anal SIL but not with detection of anal HPV.
VULVAR, VAGINAL AND ANAL INTRAEPITHELIAL HIGH-GRADE-NEOPLASIA IN HIV-POSITIVE WOMEN WITH MULTILOCULAR HPV-INFECTION

Hollwitz B, Böhmer G, Flemming P, Stoll M, Petry KU
Dept. Gyn., Oncology, Inst. of Pathology, Dept. Clin. Immunology, Hannover Med. School, Germany

Introduction: HIV-positive women show an increased prevalence of genital HPV-infection, cervical intraepithelial neoplasia (CIN) and invasive cervical carcinoma as well as of vulvar (VIN) and vaginal (VaIN) lesions. In our long-term-observation study on 194 HIV-positive patients we looked out for risk factors for the development of high-grade(HG)-VIN or -VaIN. Methods: The 194 pts. (age 17-69 yrs., med. 32 yrs.) were examined regularly concerning history, HIV-status, clinical examination/kolposcopy, cytology/histology, HPV-status (Hybrid Capture II, Digene). Results were matched with either pts. comparable of age with HG-CIN alone or with HPV-negative controls. The overall observation time was up to 118 months (med. 25 months); all in all 370 women-years. Results: 50 women were vulvar positive for oncogenic HPV-DNA, of whom 41 had been positive at the cervix as well. 11 women showed HG-VIN (>=VIN II), 4 of those in combination with HG-VaIN, one in combination with high-grade-AIN, two women had HG-VaIN alone, 1 HG-AIN alone, 1 had HG-VaIN with LG-VIN; 1 woman presented with low-grade-VIN+VaIN (VIN+VaIN I). 15 of these 16 pts. had also been positive for HPV-high-grade-DNA cervically in the Hybrid Capture; all 16 also suffered from CIN and showed a higher tendency of progression than the control patients. The average CD4-cell count at diagnosis was 233/µl in the pts. with multilocular lesions, 386 in those with high-grade-CIN alone and 452 in the HPV-negative controls. Conclusion: Vulvar and vaginal intraepithelial neoplasia occur frequently in HIV-infected women and go along with cervical high-grade lesions – often rapid progressive – and poorer HIV-status. Cervical assessment also identifies women who are at risk for multilocular disease. Vulvoscopy/biopsy of HIV-infected women is essential.
76

PRELIMINARY STUDY ON HUMAN PAPILLOMAVIRUS TYPE 16 E6E7 DNA VACCINE AGAINST C3 TUMOR CELLS
XM Xu, MZ Zhu, JY Si, K Li, GX Song
Department of Biophysics, Institute of Basic Medical Sciences, Peking Union Medical College, Beijing, 100005 China

Background: Previous studies have unambiguously shown that HPV16 E6 and E7 are major transformational genes and play a major role in the development and maintenance of the malignant phenotype. In our study, one point mutations was introduced into the pRb-binding site of HPV16 E7 and constructed fused E6E7 genes of HPV16 as target gene of DNA vaccine by alternation of the termination coden of E6. The immunity of this DNA vaccine was evaluated in both in vitro and in vivo.

Methods: HPV16 E6E7 genes were isolated from a Chinese patient with cervical cancer and modified the E6E7 genes by introducing a mutation at the Rb binding site of E7 and creating the fused E6E7 coding sequences (designated fmE6E7). The constructed pVR1012-fmE6E7 were immunized C57BL/6 mice intramuscularly alone or associated with mouse B7-1 genes expression plasmids (pcDNA3.1-B7-1). The cytotoxic T lymphocytes (CTLs) and the specific antibody in sera was analyzed. The positive C3 tumor cells were inoculated subcutaneously in vaccinated mice to assay the growth of transplant tumors.

Results: Coadministration of fmE6E7 and B7-1 genes can significantly enhanced the CTL immune responses induced by the E6E7 genes, and protect 33% immunized mice against HPV16 positive C3 cells challenge compared with the E6E7 genes alone.

Conclusion: This result suggested that coadministration of fmE6E7 with B7-1 gene enhanced significantly the CTL immune response of fmE6E7 and could provide a way to developing a more effective therapeutic vaccine for the tumors.
THE RELATIONSHIP OF HAART USE AND THE REGRESSION OF CERVICAL SQUAMOUS LESIONS AMONG HIV-INFECTED WOMEN

LA Grant1, R Li1, A Levine2, LS Massad3, H Strickler4, H Minkoff5, M Moxley6, J Palefsky7, H Sacks8, SJ Gange1. 1Johns Hopkins Bloomberg School of Public Health, 2Univ of Southern California School of Med, 3Southern Illinois Univ School of Med, 4Albert Einstein College of Med, 5Maimonedes Med Ctr, 6Georgetown Univ Med Ctr, 7Univ of California, San Francisco, 8Mount Sinai Med Ctr

Background: Immunosuppressed HIV+ women have an elevated risk of cervical HPV infection and persistent squamous intraepithelial lesions (SIL). The association between immunosuppression and the regression of cytological lesions in the context of highly active antiretroviral therapy (HAART) has not been fully evaluated. Methods: 312 HIV+ participants in the Women’s Interagency HIV Study, a multi-center cohort study, with incident cervical cytological lesion (low grade SIL or above, LSIL+) were followed for evidence of cytological regression. Pap smears, CD4+ cell counts, and HAART were evaluated every 6 months. Follow-up time contributed prior and subsequent to HAART initiation was classified as before and after HAART initiation, respectively. Women who were treated for cervical disease were censored at the time of treatment. The outcome measure of interest was lesion regression, defined as 2 consecutive normal smears. Incidence rates of lesion regression were computed among person-years at risk both prior to and subsequent to HAART initiation. Results: A total of 141 women regressed to normal cytology. Compared to regressors, non-regressors had lower CD4+ cell counts (230 versus 336, p<0.01) and were more likely to have prior ASCUS (64.9% versus 51.1%, p=0.02) at incident LSIL+. Among HAART initiators, the incidence of regression before and after initiation, respectively, was 0% (95%CI: 0, 2.4) and 12.5% (95%CI: 9.9, 15.1) and regression following HAART initiation was related to CD4+ cell count (p for trend=0.002). Conclusions: Our results indicate that HAART use was associated with increased incidence of regression to normal cytology and that among HAART initiators, elevated CD4+ cell counts were associated with a greater risk of regression.
78

LOCAL IMMUNE RESPONSE IN ANAL PRE-INVASIVE LESIONS RELATED TO HPV SEROTYPES

J. Mullerat¹, B. Lloveras², W. Quint³, M. C. Winslet¹, M. Bofill⁴

University Department of ¹Surgery, ⁴Immunology, Royal Free and University College Medical School, London, U.K, ²HPV Laboratory, Institut Catala d’Oncologia, L’Hospitalet, Barcelona, Spain, ³Delft Diagnostic Laboratory, The Netherlands.

Several studies have demonstrated that anal canal infection with carcinogenic strains of human papillomavirus (HPV) is a requirement for the development of anal squamous intraepithelial lesions (ASIL) and anal squamous cell carcinoma (SCC). Since the AIDS epidemic, the incidence of anal SCC in immunosuppressed homosexual males has increased by almost x100. It remains unclear whether this increase is due to the patient’s immunosuppression or to the HPV virulence.

This study assessed the degree of local T lymphocytic infiltrate (CD3) in 75 patients (41 HIV – and 34 HIV+) with histological diagnosis of HPV related anal lesions (anal warts, AIN and anal SCC) and the HPV serotype/s of these lesions was determined by PCR analysis of carcinogenic HPV strains (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) using SPF-10 primers and LiPA.

In HIV – patients, this study showed a 10 -12 fold increase in local T lymphocytic infiltrate compared with normal skin, while in HIV + patients the increase was 5 – 7 fold. 74% of lesions in the HIV – group and 88% in the HIV + group were associated with carcinogenic HPV types.

Despite a more aggressive progression to HG-AIN and anal SCC and a higher exposure to carcinogenic HPV types, HIV + patients show a significantly lower local immune response.

These data suggest that the poorer prognosis in HIV + patients is primarily due to the inherent cellular immunosupression of this group, rather than a higher exposure to carcinogenic HPV types.
LOCAL IMMUNE RESPONSE IN ANAL HPV RELATED LESIONS. COMPARISON BETWEEN HIV POSITIVE AND HIV NEGATIVE POPULATIONS

J. Mullerat\textsuperscript{1}, A. Condez\textsuperscript{2}, S. E. Davies\textsuperscript{3}, M. C. Winslet\textsuperscript{1}, M. Bofill\textsuperscript{2}
University Department of \textsuperscript{1}Surgery, \textsuperscript{2}Immunology and \textsuperscript{3}Histopathology, Royal Free and University College Medical School, London, U.K.

Introduction: The incidence of anal squamous cell carcinoma (SCC) has increased 100-fold in some groups since the AIDS outbreak. Most cases of anal carcinoma develop from high grade anal intraepithelial neoplasia (AIN) caused by persistent human papillomavirus (HPV) infection. We studied the host’s local cellular immune response in pre-invasive anal lesions and compared the results between HIV + and HIV - groups.

Method: Multiple resection specimens from 60 patients (31 HIV +) with anal warts (14), low grade AIN (12), high-grade AIN (22) and anal SCC (12) were studied. The samples were stained with CD-3, CD-4 and CD-8 antibodies. Stromal and epithelial infiltrates were counted and compared between groups. 8 patients with normal anal skin were used as controls.

Results: There was a local lymphocytic infiltrate in the stroma and epithelium, in all groups of lesions, in both populations. The T cell infiltrate (CD4 and CD8) showed a significant (p<0.05) increase as the lesions became gradually dysplastic and invasive both in HIV – and HIV + groups. The cell infiltrate in HIV+ was significantly lower in the HIV + group for each group of lesions. The CD4:CD8 ratio reversed in the HIV + group.

Conclusion: This is the first study that shows a local cellular immune response in anal HPV lesions. The progression of the lesions towards dysplasia and anal SCC is associated with increased lymphocytic epithelial and stromal infiltrate, with a progressive increase of the CD4:CD8 ratio. This explains why immunosuppressed individuals have higher risk of their lesions progressing towards anal SCC.
COMBINED MODALITY THERAPY FOR INVASIVE ANAL CARCINOMA IN HIV-INFECTED PATIENTS (PTS) IN THE ERA OF HIGHLY ACTIVE ANTIRETROVIRAL THERAPY. M Ferfolia, J. Orem, J Ambrose J Jasper, H Reynolds, T Kinsella, and SC Remick. Departments of Medicine, Surgery, Radiation Oncology and the Comprehensive Cancer Center at University Hospitals of Cleveland and Case Western Reserve University, School of Medicine, Cleveland, OH.

Invasive carcinoma of the anus in HIV-infected individuals is seen in increased incidence (JNCI 92:1500, 2000), though late-stage cancer invasion is not linked to degree of immunosuppression (JAMA 285:1736, 2001). Given this observation, as pts with HIV infection live longer in the HAART era it can be anticipated that invasive anal carcinoma will be an increasing problem. In our own institution we have seen an increase in this disease and report our experience with combined modality therapy. Since 1999 (4 pts in 2002) we have treated 8 pts (7M) with invasive anal carcinoma with combined modality therapy: mitomycin-C 10 mg/m² IV push and 5FU 1000 mg/m²/d as a 96-hour continuous infusion both at the start (day #1) and at the beginning of the fifth (day #29) and final week of radiation (1.8 Gy fractions to a total dose of 50.4-54.0 Gy) in the majority of cases. Six of 8 pts had prior AIDS with median CD4 lymphocyte count 137 (range: 30-600/µL) and HIV-1 viral load 17,556 (range: undetectable-114,000 copies/mL); and stage of disease: 4 pts T₁NₓM₀; 1 T₂N₀M₀; 2 T₃NₓM₀; and 1 T₂N₂M₀. At the completion of treatment all pts underwent thorough anoscopy and response evaluation. Of 7 cases that have completed therapy, 6 had CR (including 1 unconfirmed CR) and 1 PR; toxicity has generally been mild with only 2 cases of ≥ grade 3 myelo-suppression; 3 pts with grade 2 skin (perineum) toxicity; and 3 pts with grade 2 diarrhea. A diverting colostomy was performed in a single pt at time of diagnosis; 2 others had a colostomy placed at time of relapse in one and another with intractable pain syndrome thought attributable to recurrent disease with subsequent negative pathological confirmation. Two pts died at 18 and 20 mos. after therapy with disease progression; 5 are alive without relapse at 2+, 8+, 10+, 14.5+ and 37+ mos.; and one pt lost to follow-up. In summary, combined modality therapy of invasive anal carcinoma in this setting is very well tolerated with acceptable durable response and sustained remission rates.
THE INTERACTION BETWEEN HIV-1 TAT AND PRB2/P130: A POSSIBLE MECHANISM IN THE PATHOGENESIS OF AIDS-RELATED NEOPLASMS

Giulia De Falco, Cristina Bellan1, Stefano Lazzi1, PierPaolo Claudio2, Caterina Cinti3, Piero Tosi1, Antonio Giordano2 and Lorenzo Leoncini1. 
1University of Siena, Siena, Italy; 2Temple University, Philadelphia, PA; 3CNR, Bologna, Italy.

HIV-1 has long been recognized as the etiological agent of acquired immunodeficiency syndrome (AIDS). Although many neoplasms arise in HIV-1 infected patients more frequently than in other forms of immunosuppression, the role of HIV-1 as an oncogenic virus has not yet been clarified. The HIV-1 gene product Tat, secreted by HIV-1 infected cells and taken up by normal cells, is a likely candidate to contribute to tumor pathogenesis in HIV-1 infected patients because of its growth promoting activity, angiogenic function and antiapoptotic effect. The oncogenic role of Tat is further supported by the development of non-Hodgkin's lymphomas in Tat-transgenic mice. Furthermore, a virus-linked mechanism of lymphomagenesis, in AIDS-related lymphomas has recently been proposed, involving the RB2/p130 pathway. The absence of mutation in the RB2/p130 gene and the unusually high percentage of cells expressing pRb2/p130 in tumors with a high proliferative activity such as AIDS-related lymphomas, may in fact suggest a physical interaction of pRb2/p130 with viral oncoproteins. However, little is known about the mechanism by which HIV-1 gene products interact with RB family and other cell cycle regulatory proteins. The aim of our study was to investigate whether Tat could bind to pRb2/p130, thus impairing its tumor suppressor activity. Our results show that the two proteins interact both in vitro and in vivo, through the pocket region of pRb2/p130. Tat seems to inactivate the tumor suppressor activity of pRb2/p130, as demonstrated by a colony assay. In addition, we observed that Tat does not compete with E2F-4 in binding to pRb2/p130. Due to the overexpression of pRb2/p130 observed in AIDS-related lymphomas, we investigated whether Tat could influence either the phosphorylation status of pRb2/p130 or its expression at mRNA level. Our results show that Tat does not alter the phosphorylation status of pRb2/p130, but increases its expression at mRNA level. The interaction between Tat and pRb2/p130 may lead to a deregulation of cell growth control by Rb-related proteins, that may contribute to lymphomagenesis in AIDS-related patients. The understanding of basic information may be of significance for prognosis and implementing future therapeutic regimens, including the design of novel therapeutic approaches. As a matter of fact, spontaneous regression of HIV-1 associate lymphoproliferative disorders has been reported following highly active antiretroviral therapy.
DISSOCIATED EXPRESSION OF IFN-α AND OF GRANZYMЕ-B BY HIV SPECIFIC CD8+ CELLS IN INFECTED INDIVIDUALS. To Kleen, C Kruse, PV Lehmann and M Tary-Lehmann. Case Western Reserve University, School of Medicine, Dept. Of Pathology, Cleveland, OH 44106, USA. Unlike naive T cells that do not express IFN-α or Granzyme B, memory CD8 cells rely on these molecules for mediating different effector functions. By secreting IFN-α, CD8 cells locally activate macrophages to engage in host defense. Granzyme B, in contrast, is part of the cytolytic machinery that CD8 cells utilize for perforin-mediated killing of their target cells. In our first set of experiments we show here that immunizations with different adjuvants can induce in mice CD8 cells that differentially express these molecules. Immunization with MHC class I restricted peptides in complete Freund’s adjuvant induces IFN-α producing CD8 cells that do not mediate cytotoxicity in chromium release assays. In contrast, injection of the same peptide, in the same dose, but in incomplete Freund’s adjuvant, induces CD8 cells that kill, but that do not produce IFN-α (or type 2 cytokines). The former CD8 memory cell type mediates delayed type hypersensitivity, the latter does not induce it. Therefore, in mice, the two discrete CD8 cell memory subpopulations exist whose differentiation relies on different signals, and that serve different effector functions, DTH vs. cognate cytolyis. In our second set of experiment, we tested whether also in humans there is evidence for such differential CD8 cell populations. We studied HIV infected individuals, using HIV peptides to elicit the specific CD8 cells. IFN-α and Granzyme B ELISPOT assays were performed. We found that some peptides induced only IFN-α, in the absence of Granzyme B, while other peptides induced Granzyme B production by CD8 cells, in the absence of IFN-α. The data provide evidence that also in humans these two CD8 subpopulations exist. Because of the different effector functions that they exert, it will be important to establish their relative contribution to anti-HIV immunity. Similarly, when studying immunity induced by HIV vaccines, it should be critical to account for the Granzyme B producing CD8 cell type as well, in addition to the conventionally studied IFN-α secreting CD8 cells.
DOWN-REGULATION OF CELL CYCLE CHECKPOINT PROTEIN, P27 (KIP 1) IN CD8+ CELLS OF HUMAN IMMUNODEFICIENCY VIRUS TYPE-1 INFECTED SUBJECTS ON HIGHLY ACTIVE ANTIRETROVIRAL THERAPY

Eva M. McGhee, Leyla S. Diaz, and Hillary G. Foster.
University of California San Francisco.

Previous studies have shown that changes observed in human immunodeficiency virus type-1 (HIV-1) infected peripheral blood monocytic cells may be involved in abnormal cell cycle regulation, the development of HIV-1-related malignancies, and increased apoptosis. Cell cycle regulatory controls occur at G1/S or G2/M boundaries and provide for an ordered entry into S or M phase. Such checkpoints allow for repair of DNA damage prior to the initiation of replicative DNA synthesis or cell division. Alternatively, in instances of extensive genomic damage, cells may commit to a pathway of apoptosis or programmed cell death. We are currently investigating genomic damage and cell cycle regulatory events in HIV-1 infected cells from subjects receiving highly active antiretroviral therapy (HAART). When HIV-1 infected cells are treated with gamma irradiation, these cells lose their G1/S checkpoints, enter the S phase inappropriately, and eventually apoptosis. The loss of G1/S checkpoints, is associated with a reduction in cell cycle proteins p21/Waf1 and p53, and has been linked to secondary cancers. We show that p27 (KIP1) a regulatory inhibitory protein in the cell cycle is down-regulated in CD8+ cells from subjects receiving HAART. cDNA from CD8+ cells of HIV-1 infected subjects receiving HAART, those no longer on HAART, and uninfected people were analyzed by microarray analysis to determine gene expression of the cell cycle protein, p27 (KIP1). Microarray analysis for gene expression indicated that p27 (KIP1) down regulated in CD8+ cells from HIV-1 infected subjects on HAART when compared to people that are un-infected, and subjects infected but not on HAART. Down regulation of the cell cycle regulatory protein p27 (KIP1) in cells from HIV-1 infected subjects on HAART may pose obstacles for effective optimal treatment in HIV-1 patients. Therefore, we are analyzing additional CD8+ cells from subjects receiving HAART, and using RT-PCR to determine protein expression of other proteins that may be involved in abnormal cell cycle regulation. This information can be helpful in advancing our knowledge of cell cycle regulatory proteins in HIV-1 and associated malignancies, and could lead to new strategies for better therapy.
84

EPIDEMIC SITUATION OF HIV INFECTION IN UKRAINE

Ruslan Sandulyak, Chernivtsy Regional Oncology Center

Since 1995, the number of HIV-infected people in Ukraine has increased dramatically. According to the Ukrainian Center for AIDS Prevention, more than 50,000 people are registered with HIV/AIDS, and up to an estimated 400,000 people are infected, the majority of whom are youth who contracted the virus via injecting drug use and approximately 1% of the adult population. Situation analyses have shown that injecting drug users, commercial sex workers, prisoners and people in uniform are most vulnerable to infection in general.

The analysis of the dynamics of the epidemic process for 15 years made it possible to find out the presence of too separate epidemic waves of HIV infection. The first wave (1987-1994) was manifested as the slow type of the development of the epidemic, characterized mainly by sexual transmission. During this period 402 persons with HIV infection were detected, 24 persons were found to have AIDS; of these, 13 persons died. The second epidemic wave began in 1995 and was due to the spread of HIV among users of drugs introduced by injection. By the end of 1995 the number of HIV carriers was 34 times greater than that of 1994, reaching 1490 persons. In 1996-1997 this figure increased 8 times (annually). The number of AIDS patients rose to 420 persons. And now, we have more than 50,000 people with HIV/AIDS.

We observed 345 hospitalized patients, aged mainly 17-36 years (40 patients with active AIDS, 43 patients with AIDS, other patients were HIV carriers and infected at the stage of lymphadenopathy). In most of the HIV-infected patients the infection process progressed in 3-5 years, which was manifested by associated candidosis in 74.7% of cases. In AIDS patients opportunistic infections of viral etiology (herpes simplex, cytomegalovirus infection, etc.) prevailed. 14 patients were found to have tuberculosis. Malignancies were diagnosed in 27 patients with AIDS. Among them there were 15 cases of Kaposi’s sarcoma, 6 patients with invasive carcinoma of the cervix, 2 with testicular cancer, 2 with brain lymphoma, 1 with melanoma and 1 with Hodgkin’s disease. Progressive cytopenias (anemia, thrombocytopenia, leukopenia) were occurred in major of HIV-infected patients. Maybe it was coursed by myelosuppressive effects of antiretroviral, anti-infective, and antineoplastic therapies.

Clinico-epidemiological analysis made it possible to come to the conclusion that the specific features of HIV carriership and AIDS were greatly linked with different groups of risk to which the patients belonged. Thus, a shorter period of carriership, the prevalence of opportunistic viral infections were mostly characteristic of drug addicts.
HIV SERO-POSITIVITY IN SURGICAL PATIENTS IN A NIGERIAN TEACHING HOSPITAL – A MANAGEMENT CHALLENGE

Dr I N Usoro and Dr Iquo Ibanga
University of Calabar Teaching Hospital, Nigeria.

This study was aimed at identifying what proportion of HIV sero-positive patients in University of Calabar Teaching Hospital (UCTH) was from Surgery Department, their Age and Sex distribution, and the challenges in managing these patients. The records of the HIV Laboratory in Haematology Department, UCTH were examined retrospectively between January 1999 and December 2002 and compared with records in the Surgical Wards, Surgical Outpatient Department, and Casualty Department. Name of patient, age, sex, Hospital Number, Ward, Serology Kits used, and results were extracted there from. We recorded a total of 992 HIV sero-positive patients for the period. All patients had been tested with at least 2 kits and a 3rd kit used in cases of doubt; ELISA type and Latex/Colloid Agglutination methods were used. 228 patients (23%) were from Surgery Department. The youngest was 8 months and the oldest 81 years old. Peak age group was 21-30 years (63 cases or 27.6%). Overall Male to Female (M:F) ratio was 1.1:1 but females predominated in age group 21-30 years (M:F 1:1.2) and in an unspecified group of “Adults” (M:F 1:1.3). Highest M:F ratio was recorded in age group 51-60 years (4:1). There was no evidence of consistent follow-up or treatment. We concluded that a significant proportion of HIV sero-positive patients in UCTH are from Surgery Department, apparently due to routine testing. The “African phenomenon” of near–equal M:F ratio is present here. There is an urgent need for a formal protocol for the management of these sero-positive patients, many of whom are “incidental findings”. Without facilities for CD4 Cell Count, HIV Viral Load assay, and drug monitoring (for effectiveness and toxicity), Antiretroviral medication constitutes near-blind therapy, and may even do more harm than good in some cases. Scientists worldwide need to examine ways of extending the benefits of current knowledge of HIV diagnosis and management to patients in the developing world. For example, tying aid and grants to collaboratory scientific studies would likely minimize political and bureaucratic interference. A prospective study is needed to answer more questions on prevalence, sensitivity of diagnosis, and management.
AIDS PATIENTS HAVE HIGH LEVELS OF PROTEIN NITRATION WITHIN ACTIVELY HIV INFECTED CELLS. DOES THIS CAUSE ORGAN MALFUNCTION OR CANCER?

Leona W. Ayers¹, Michael J Mihm², Barbara Hackman³, John Anthony Bauer⁴. The Midregion AIDS and Cancer Specimen Resource (ACSR) ¹,³, Department of Pathology, and Division Developmental Pharmacology, Children's Research Institute²,⁴, The Ohio State University, Columbus, OH 43210.

Evidence suggests that reactive nitrogen species or derivatives induced by infection underlie carcinogenesis. Of particular biological significance is peroxynitrite that associates with protein nitration, DNA-strand breakage, and quanine nitration causing cell malfunction as well as mutagenesis. Pre-HAART HIV infected patients demonstrate significant organ malfunction and increased rates of all types of malignancies. In post-HAART HIV infected patients both organ dysfunction and malignancies have declined with documented declines in post treatment virus blood levels.

Methods: Tissue micro-arrays (TMAs) of 0.6 mm and 2.0mm were constructed of autopsy tissues from a population of pre-HAART AIDS patients with cardiomyopathy, dementia, hepatitis, lymphoma, etc. Test results and associated patient demographic and clinical data were managed by a Microsoft Access® database.

Results: Nitrotyrosine immunostaining for protein nitration was prominent in the cells of dysfunctional organs along with PCNA staining macrophages. Conclusions: Increased levels of protein nitration and significant levels of virus are associated with clinical organ malfunction. Associated mutagenesis in susceptible tissues remains of interest. Information about the national ACSR and the types of tissues banked is available at http://acsr.ucsf.edu. Specific interest in AIDS-related malignant TMAs can be directed to the OSU-ACSR, Leona W. Ayers, M. D., M352 Starling Loving Hall, 320 W. 10th Avenue, Columbus, Ohio 43210, (614-293-8106).
THROMBOSIS IN HIV-INFECTED PATIENTS IN THE ERA OF HAART

Michael C. Jacobson,1 Bruce Dezube, MD,2 and David M. Aboulafia, MD,3 1University of Washington School of Medicine, Seattle, WA, 2Beth Israel Deaconess Medical Center, Boston, MA and 3Virginia Mason Medical Center, Seattle, WA.

Prior to HAART, HIV-related thrombotic complications were rarely reported. Increasingly common are cases of HIV patients presenting with unprovoked thrombosis. It is unclear whether thrombosis occurs as a result of HIV itself, medications, or various infections. We retrospectively reviewed data from a clinic of 600 HIV patients seen between 1996-2002. 25 male patients had DVT, PE, myocardial infarction (MI), or cerebral vascular accident (CVA). For each thrombotic event we recorded CD4+ count, HIV VL, and, for the majority, a detailed thrombophilia evaluation. 20 patients (80%) were smokers (median; 22 pack-years). 18 patients (72%) had a single thrombosis while 7 patients (28%) had >2 distinct events (range 1-4). 12 patients (48%) had leg DVT, 10 (40%) had PE (2 noted at autopsy), 4 (16%) had CVA, 6 (24%) had MI, 1 (4%) had a catheter-associated subclavian DVT, and 1 had splenic DVT. The median CD4+ count at time of thrombosis was 328 cells/μL (range 7-800), and the median HIV VL 1280 copies/mL (range 50-700,000). 17 patients (68%) were on HAART at time of thrombosis. 12 (48%) patients were diagnosed with cancer (8 KS, lung, prostate, NHL and Hodgkin’s). 16 patients (64%) had significant dyslipidemia. 2 patients with PE had antiphospholipid syndrome. Thrombosis was associated with a wide range of HIV disease states, was strongly linked to malignancy and dyslipidemia, and was heralded in 2 cases by presence of a lupus anticoagulant. Controlled studies to investigate the relationship of abnormalities in thrombophilia workup to actual presentation with thromboembolism are needed in this diverse patient group.
EFFECTS OF ALCOHOLISM AND HIV STATUS ON ALCOHOL-RELATED CANCERS AMONG VETERANS.
Bryant, K1, Roach, D1, McGinnis, K2, Skanderson, M.2, Conigliaro, J2, Justice, A2 and VACS Project Team2. National Institute on Alcohol Abuse and Alcoholism (NIAAA) 1, Veteran’s Administration (VA)2 Known immunosuppressive effects of both HIV and alcohol suggest possible parallels in the pathogenesis of malignancies associated with AIDS and alcohol dependence. Animal research suggests that alcohol, an environmental risk, promotes tumor growth, particularly in the liver. Cohort and case-control epidemiological research has associated harmful alcohol consumption with increased risk of specific cancers. Strong relationships have been found between alcohol and cancer of the liver, oral cavity, pharynx, larynx, and esophagus. More moderate relationships have been found between alcohol consumption and cancer of the large bowel and breast. Clinical outcomes research supported by NIAAA, in collaboration with the VA, focuses on the interaction of alcohol consumption, HIV, and associated comorbidities. The present epidemiological study included 49,299 HIV+ and HIV- Male and Female Veterans between the ages of 25 and 84. Individuals were identified as HIV+ (n=24,897) or age matched HIV- controls (n=24,402). The groups differed in rates of alcohol diagnoses (ICD 9 codes): 21.8% HIV+ vs. 17.2% HIV- (p<.001). Analyses for the impact of HIV and alcohol consumption on selected cancers were carried out using Poisson regression adjusted for age and race categories. HIV positive patients and alcoholics have more cancers (p<0.001). For liver cancer: cancer is positively associated with HIV (p=0.023) and a diagnosis (dx) of alcohol dependence (p<0.001). For oral cancer: HIV dx is not significantly associated (p=0.275), but alcoholism dx is (p<0.001) For stomach cancer: neither HIV nor alcohol dx are associated. For lung cancer: HIV dx is positively associated (p=0.001) and alcohol dx shows a trend (p=0.156) For bladder cancer: neither HIV nor alcohol are significant. For prostate cancer: HIV and alcohol look protective (p<0.001 and p=0.079). The patterns of additive effects of alcohol and HIV dx have significant implications for underlying biological and behavioral interactions and possible preventive interventions.
We report in an adult with a positive HIV serology, the case of a MALT lymphoma remission induced by the exclusive use of antiretroviral treatment.

A 63-year-old female was admitted with a history of cough, dysphonia and weight loss for which investigations has revealed a positive HIV serology and a mediastinal mass on chest CT scan. A bronchoscopy showed two bronchic inflammatory stenosis with on biopsy a lymphomatous proliferation with a lymphomatoplasmodicratory differenciacion with a kappa monotypy. This aspect on this place has suggested the diagnosis of MALT lymphoma. The screening of the disease revealed a laryngeal disease and 3 conjunctival masses on the left eye. There was no adenopathy on abdominal CT scan, and digestive endoscopy were normal. Blood immunologic lymphocyte phenotype study showed a Kappa predominance (85%) and immunoelectrophoretic migration revealed a monoclonal M immunoglobulin and a cryoglobulinemia was detected. The bone marrow biopsy showed a 5-10% interstitial and nodular lymphoid infiltration CD20+. Initial immunovirologic staging for HIV was 228 CD4 lymphocytes / μl (27%) with 222,213 HIV RNAcopy/ml viral load. HCV serology was negative. Antiretroviral therapy was initiated with AZT-3TC and Nevirapine (2 months). It was changed a first time for D4T-3TC and Nevirapine ( 2 months) because of an anemia occurrence, and a second time for 3TC-Tenofovir and Nevirapine for a clinical and biological pancreatitis.

Three months after antiretroviral therapy was started laryngeal and conjunctival masses has disappeared as mediastinal mass on chest CT scan. Three bronchic biopsies has been done in the same first site studied. There was no lymphoid infiltration but FR2 clonality was discretely positive with a very low signal. Monoclonal M immunoglobulin has disappeared. Blood HIV viral load was undetectable (positive for 20 ARN copy/ml) with 182 CD4 lymphocytes/μl. At 6 months the patient was asymptomatic with a normal chest CTscan, a positive cryoglobulinemia, an undetectable HIV viral load and 306 T4 lymphocytes/μl.

This case report suggests that Malt Lymphoma may be induced by the way of the direct or indirect HIV mediated B cells polyclonal activation.
FAILURE OF AIDS-ASSOCIATED PRIMARY EFFUSION LYMPHOMA (PEL) TO RESPOND TO HIGH-DOSE CHEMOTHERAPY (HDC) AND AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT)

David M. Aboulafia, MD, Virginia Mason Medical Center, Seattle, WA.

PEL is a newly recognized AIDS-related malignancy that is etiopathologically linked to Kaposi’s sarcoma (KS)-associated human herpes virus-8 (HHV-8). PEL is characterized by presentation in serous body cavities without identifiable tumor masses. PEL cells have high-grade morphological features, an indeterminate immunophenotype, B-lineage genotype, and contain HHV-8 and often EBV. PEL rarely responds to chemotherapy. A 48-year-old man with a long history of AIDS, a CD4+ count of 300 cells/µL and an HIV viral load < 50 c/µL was receiving paclitaxel for progressive KS when he developed an exudative right pleural effusion. Thoracentesis revealed immunoblastic cells. Flow cytometry showed a prominent population of bright CD45 and CD38+ cells with no significant CD29, CD56, or CD20 expression. Southern blot confirmed a B-cell genotype. Formalin fixed deparaffinized sections were incubated with monoclonal and polyclonal antibodies for HHV-8 and EBV and the neoplastic nuclei were positive for HHV-8-associated latent protein and EBV. He received EPOCH chemotherapy x 2 followed by salvage ifosfamide, carboplatin and etoposide (ICE) with G-CSF and stem cell collection. Subsequently, he received HDC consisting of cyclophosphamide 3 gm/m² intravenously on days 1 and 2 and oral busulfan 15 mg/m² per day x 4 days in divided doses. Treatment was well tolerated but the tumor remained chemo-refractory and he succumbed to progressive cancer 1 month post ASCT. Treatment for patients with PEL remains imperfect; their tumors rarely respond to conventional chemotherapy. Although ASCT is a promising treatment for some HIV-associated NHLs, our experience with this patient serves to underscore the high mortality rate associated with this unique neoplasm.
SUCCESSFUL THERAPY OF RELAPSED HIV-RELATED PRIMARY EFFUSION LYMPHOMA (PEL) WITH HAART AND RITUXAN

Nancy Rubin1, Jonathan Said2, Alexandra M. Levine1. 1University of Southern California; 2University of California in Los Angeles, Los Angeles, California.

Background: PEL is associated with infection by HHV8/KSHV, and has been described in both HIV positive and negative patients. In the setting of HIV infection, the prognosis has been poor, with median survival in the range of 60 days. Optimal therapy is unknown.

Methods: We reviewed the case records of an HIV + patient with relapsed PEL. Assessment of HHV8 was made by immunohistochemistry (IHC) in non-Hodgkin’s lymphoma (NHL) tissue at original diagnosis and at relapse.

Results: The patient was a 44 year-old male, HIV + since 1982. Diagnosis of diffuse large B-cell NHL made in 1998, stage IE, with isolated involvement of the bowel; CD20+. Therapy with CHOP resulted in CR, lasting 3 years. In 2/02, the patient presented with cardiac tamponade with a large pericardial effusion, which revealed classic morphology of PEL, CD20 negative. He has been on no anti-HIV therapy. CD4+ cells were 173/dl and HIV-RNA was 1398 copies/mL. Tamponade was drained, and patient begun on HAART (nelfinavir, efavirenz, and lamivudine) with weekly rituximab, resulting in a CR of the pericardial effusion. CD4 cells rose to 244/dL and HIV-RNA was <50 copies/mL. There was no further evidence of PEL. Three months later, the patient was in an auto accident, and subsequently died. Autopsy revealed no evidence of lymphoma. IHC of the original bowel NHL revealed HHV8.

Conclusions: PEL may be effectively treated with HAART + rituximab in HIV infected patients.