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High Prevalence of Epstein–Barr Virus in the Reed–Sternberg Cells of HIV-Associated Hodgkin's Disease

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The Epstein–Barr virus (EBV) has been implicated in the pathogenesis of Hodgkin's disease, with an frequency of 15 to 50% in the immunocompetent host. We studied 12 formalin-fixed, paraffin-embedded cases of Hodgkin's disease occurring in human immunodeficiency virus–infected individuals to determine the frequency of EBV in Hodgkin's disease from this population. EBV DNA-RNA in situ hybridization was performed using a 30-base biotinylated anti-sense oligonucleotide complementary to the EBER1 gene of EBV. EBV RNA was found in the Reed–Sternberg cells and variants in 11 of 12 cases. Double-labeling studies confirmed the presence of EBV RNA in CD15-expressing Hodgkin's cells in all 11 cases, although rare B lymphocytes coexpressing EBV RNA and CD20 were also noted in these cases. The Hodgkin's cells in all 11 EBER-positive cases expressed latent membrane protein. The one case negative for EBV RNA showed the histology of nodular, lymphocyte predominance, a subtype thought to be distinct from other types of Hodgkin's disease. (Am J Pathol 1993, 142: 1073–1079)

The pathogenesis of Hodgkin's disease is controversial, particularly regarding the role of the Epstein–Barr virus (EBV). EBV genomic DNA has been demonstrated in involved tissues from patients with Hodgkin's disease,[1–4] and *in situ* hybridization methods have localized the virus to Reed–Sternberg cells and variants (Hodgkin's cells), the presumed neoplastic cellular component.[3–6] Whereas initial studies suggested that about 20% of Hodgkin's disease cases were associated with EBV,[1–4] recent analyses utilizing more sensitive immunohistochemical and *in situ* hybridization techniques have demonstrated EBV within Reed–Sternberg cells in about 50% of cases of typical Hodgkin's disease occurring in immunocompetent individuals.[5,7,8] These latter studies have also localized EBV to rare reactive B and T lymphocytes in a majority of cases.[5]

Evidence of EBV has been found in approximately 50% of cases of human immunodeficiency virus (HIV)-associated non-Hodgkin's lymphomas.[9,10] In addition, the presence of EBV genomes in benign lymph node biopsies from HIV-infected patients may be the initial source of EBV-infected malignant B-cell clones or even a source of superinfection or second-hit of already established B-cell neoplasms.[11,12] However, the existence of malignant lymphomas unassociated with EBV makes the exact pathogenetic role of the herpes virus difficult to define.

Several investigators have proposed that HIV-infected individuals may have a higher incidence of Hodgkin's disease, although this increase is certainly not of the order of that of Kaposi's sarcoma or non-Hodgkin's lymphoma in AIDS patients.[13,14] In the current study, we have investigated the presence of EBV in HIV-associated Hodgkin's disease. Using the technique of *in situ* hybridization, we distinguish EBV within the Reed–Sternberg cells and variants from EBV within small lymphocytes, probably due to the immunosuppression present in these patients. Our studies reveal that all 11 cases of classical Hodgkin's disease occurring in HIV-infected patients contained EBV within the Reed–Sternberg cells and variants, suggesting an important role for EBV in the pathogenesis of this neoplasm.

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Materials and Methods

Cases

Twelve formalin-fixed, paraffin-embedded cases of Hodgkin's disease were analyzed. The cases were derived from the files of the four affiliated hospitals of the University of California at San Francisco. The patients had documented AIDS, documented AIDS-related complex with HIV-positivity, or in one case, an extremely high suspicion of HIV-positivity before the establishment of the diagnosis of Hodgkin's disease.

In Situ *Hybridization Studies*

The EBV RNA in situ hybridization studies were performed using a 30-base oligonucleotide complementary to the portion of the EBER1 gene, a region of the EBV genome that is actively transcribed (up to 10^7 copies per cell) in latently infected cells.[15] The oligonucleotide was biotinylated using methods previously described.[16] The procedure used for the *in situ* hybridization studies has been fully described by us elsewhere.[5,16] Briefly, 10-μ sections cut from paraffin blocks of formalin-fixed tissues were deparaffinized, dehydrated, predigested with pronase, prehybridized, and hybridized overnight at a concentration of 0.25 ng/ul of probe. After washing, detection was accomplished using avidin-alkaline phosphatase conjugate followed by development of the signal with McGadey's substrate. A blue, blue-black, or black color within the nucleus over background levels was considered a positive reaction. This methodology detected EBV RNA from all cases of known EBV-positive nasopharyngeal lymphoepithelioma, acute infectious mononucleosis, and post-transplantation lymphoproliferation but showed no signal in lymphoid tissue from an EBV-seronegative individual. In addition, cells from tissues infected with herpesvirus I, papilloma virus 16, and adenovirus showed no crossreactivity. Although the sense strand oligonucleotide could not be used as a negative control (due to partial identity with adjacent anti-sense sequences), substitution of the probe with 10 other oligonucleotides of identical length and similar G-C content revealed no similar staining. Preincubation with 9 ug/ul of boiled ribonuclease A (Boehringer Mannheim, Indianapolis, IN) for 37 C overnight using buffer conditions recommended by the manufacturer, omission of the labeled probe, or addition of a 50-fold excess of unlabeled probe to the hybridization solution all caused elimination of the staining pattern. Any slide negative for EBV RNA was tested for preservation of total RNA using a poly d(T) probe as we described elsewhere.[17]

Immunohistochemical Studies

Immunophenotypic studies were carried out using a previously published technique without modification with the mouse monoclonal antibody to latent membrane protein (LMP, Dako, Carpenteria, CA) that detects a recombinant fusion protein containing sequences of bacterial beta-galactosidase and the EBV-encoded (LMP).[18,19] Normal lymphoid tissue served as a negative control for the LMP studies.

*Double-Labeling Immunohistochemical/*in Situ *Hybridization Studies*

The immunohistochemical studies were performed first, using a previously published procedure without modification,[18] with the monoclonal antibodies Leu-M1 (CD15, Becton-Dickinson, Mountain View, CA) or L26 (CD20, Dako). The *in situ* hybridization studies followed, using the same procedure outlined above.

Results

The age and sex of the patients, the previous history, the site studied, and the histological subtype of Hodgkin's disease are given in Table 1. Epstein–Barr viral RNA was identified in the Reed–Sternberg cells and variants in all 11 cases of classical Hodgkin's disease (excluding nodular lymphocyte predominance) (Figures 1 and 2). In these cases, all or virtually all of the Reed–Sternberg cells and variants were positive for EBV RNA. Double-labeling studies, successful in all 11 cases, confirmed the localization of EBV RNA to Reed–Sternberg cells and variants with the demonstration of co-positivity of the Reed–Sternberg cells and variants for Leu-M1 (CD15) and EBV RNA (Figure 3). In addition, rare, scattered smaller cells positive for EBV RNA were also identified, similar in number to what has been previously described by us in Hodgkin's disease occurring in immunocompetent individuals.[5] These cells could be distinguished from the much larger (25 to 50 μm) Reed–Sternberg cells and variants, and were generally small (7 to 15 μm). Double-labeling studies demonstrated co-positivity of many of these smaller cells for L26 (CD20) and EBV RNA, consistent with non-neoplastic B cells infected with the virus (Figure 4). These latter EBV-positive cells, although variable in number from

Table 1. *HIV-Hodgkin's Disease (Clinical)*

Patient	Age	Sex	Site	Hodgkin's disease subtype	Stage	Clinical history	Number of months from date of diagnosis
1	38	M	Retroperitoneal mass	Mixed cellularity	III B	Generalized lymphadenopathy; chronic hepatitis B infection; CMV retinitis; gonococcal proctitis; venereal warts	45.5 (Died of disease)
2	38	M	Left axillary lymph node	Mixed cellularity	IV B	ARC; gonorrhea; syphilis, shigellosis; rectal herpes; giardia; herpes genitalis; venereal warts; thrush; infectious glomerulonephritis; staphylococcal sepsis; pulmonary necrotizing granulomas	5 (Lost to follow-up)
3	32	M	Right cervical lymph node	Mixed cellularity	III A	No significant past medical history	13 (Alive)
4	21	M	Left cervical lymph node	Mixed cellularity	III A_2	Cervical lymphadenopathy	45 (Lost to follow-up)
5	36	M	Left and right tonsils	Mixed cellularity	IV	Chronic tonsillitis; Kaposi's sarcoma; MAI; anal herpes; hairy leukoplakia; thrush; basal cell carcinoma	10.5 (Died - immunoblastic lymphoma)
6	34	M	Liver and bone marrow	Nodular sclerosing, syncytial variant	IV	Lymphadenopathy; chronic perirectal abscess with fistula formation; perianal herpes; hepatitis A; herpes zoster; G6PD deficiency	0.8 (Died of disease)
7	29	M	Left cervical lymph node	Nodular sclerosing	IV B	Pneumocystis carinii pneumonia; hepatitis B; CMV pneumonia; oral herpes; thrush; scabies	38 (Alive)
8	37	M	Right axillary lymph node	Nodular sclerosing	IV B	Salmonella, shigella and cryptosporidium infections; syphilis	27.6 (Lost to follow-up)
9	30	M	Left cervical lymph node	Nodular sclerosing	III B	Hepatitis; oral herpes; syphilis; gonorrhea	23 (Alive)
10	28	M	Para-aortic lymph node	Nodular sclerosing	IV	ARC; herpes proctitis; gonorrhea; thrush; hepatitis B	15.2 (Lost to follow-up)
11	30	M	Left axillary mass	Nodular sclerosing	III	Hepatitis A and B	25 (Alive)
12	23	M	Liver	Nodular lymphocyte predominance	IV B	Sickle cell disease, transfusion dependent; delayed sexual development secondary to hypothalamic failure	98 (Alive)

CMV: cytomegalovirus; ARC: AIDS-related complex; MAI: mycobacterium avium intracellulare.

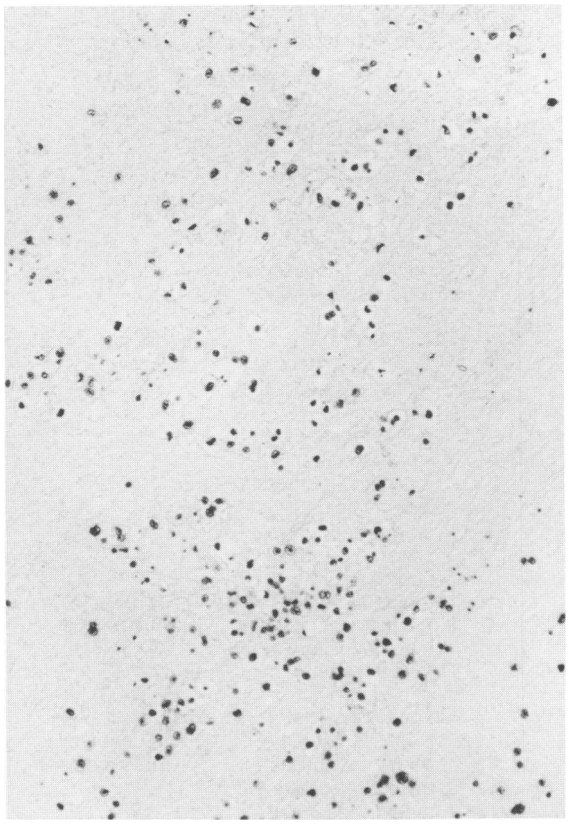

Figure 1. In situ *hybridization for EBV utilizing a biotinylated oligonucleotide probe directed against the EBER1 gene. Virtually all of the positive cells are Reed–Sternberg cells and variants. The number of positive cells varied directly with the number of Reed–Sternberg cells and variants in each case.*

case to case, comprised much less than 0.1% of the small cells, and less than 1% of the EBV-positive cells. Reed–Sternberg cells and variants showed strong membrane and cytoplasmic labeling for LMP in all 11 cases of classical Hodgkin's disease (Figure 5). Labeling was restricted to the tumor cells; small background lymphocytes did not stain with the anti-LMP antibody. In the one case of nodular L&H, lymphocyte predominance Hodgkin's disease, both L&H cells and smaller lymphocytes failed to show expression of EBV RNA. In this case, hybridization with a poly d(T) probe verified adequately preserved RNA, ruling out poor tissue processing as an explanation for the negative result.

A statistical comparison of the association between EBV and Hodgkin's disease in HIV-positive patients vs. HIV-negative patients was carried out, using the χ^2 test with continuity correction. The cases analyzed included the Hodgkin's disease cases reported in Table 1 and cases of Hodgkin's disease occurring in immunocompetent patients that were derived from our previous study that was performed using a probe and an *in situ* hybridization technique identical to that used in this study.[5] A χ^2 test with continuity correction performed on the cases of HIV-related Hodgkin's disease versus immunocompetent Hodgkin's disease showed that the difference in EBV distribution was statistically significant, $P = 0.0226$.

Discussion

In a previous study using an identical *in situ* hybridization methodology, we found 10 of 22 (45%) of cases of classical Hodgkin's disease (excluding nodular, lymphocyte predominance) occurring in immunocompetent individuals to express EBV within Reed–Sternberg cells and variants.[5] Similar results were also recently reported by Herbst and colleagues.[20] The results of the current study confirm an association of EBV with Hodgkin's disease. Moreover, we found a statistically significant difference in the percentage of cases of HIV-associated Hodgkin's disease expressing EBV within Reed–Sternberg cells as compared to Hodgkin's disease occurring in immunocompetent individuals. The uniform detection of EBV in HIV-associated Hodgkin's disease of classical histologies found in the current study suggests an important role for this virus in the pathogenesis of this neoplasm. Our results are similar to those reported by Uccini and colleagues who found evidence of EBV in 5 of 7 cases of HIV-associated Hodgkin's disease.[21] Their lower incidence of EBV positivity may have been due to their use of a less sensitive genomic EBV internal repeat probe. For example, using the internal repeat probe, they were able to identify EBV in only 3 of 20 (15%) cases of Hodgkin's disease occurring in HIV-negative individuals, much lower than the 45% incidence we obtained using the EBER1 probe.[5] Recently, two other groups reported a high frequency (8 of 10 and 2 of 2) of EBV in HIV-associated Hodgkin's disease as detected by the highly sensitive polymerase chain reaction.[22,23] However, EBV may be often detected by this method in benign lymph nodes from HIV-infected patients, presumably because of their immunocompromised state, raising questions about the significance of the findings in Hodgkin's disease without concomitant *in situ* hybridization studies to localize the EBV to the Reed–Sternberg cells.

EBV infection persists in the infected host throughout life. Presumably, immunocompetent hosts limit EBV to a low-grade active infection of oropharyngeal and salivary gland epithelial cells and a latent infection of B lymphocytes. In contrast, Birx and colleagues have shown that immunocompromised patients have a relative inability to control

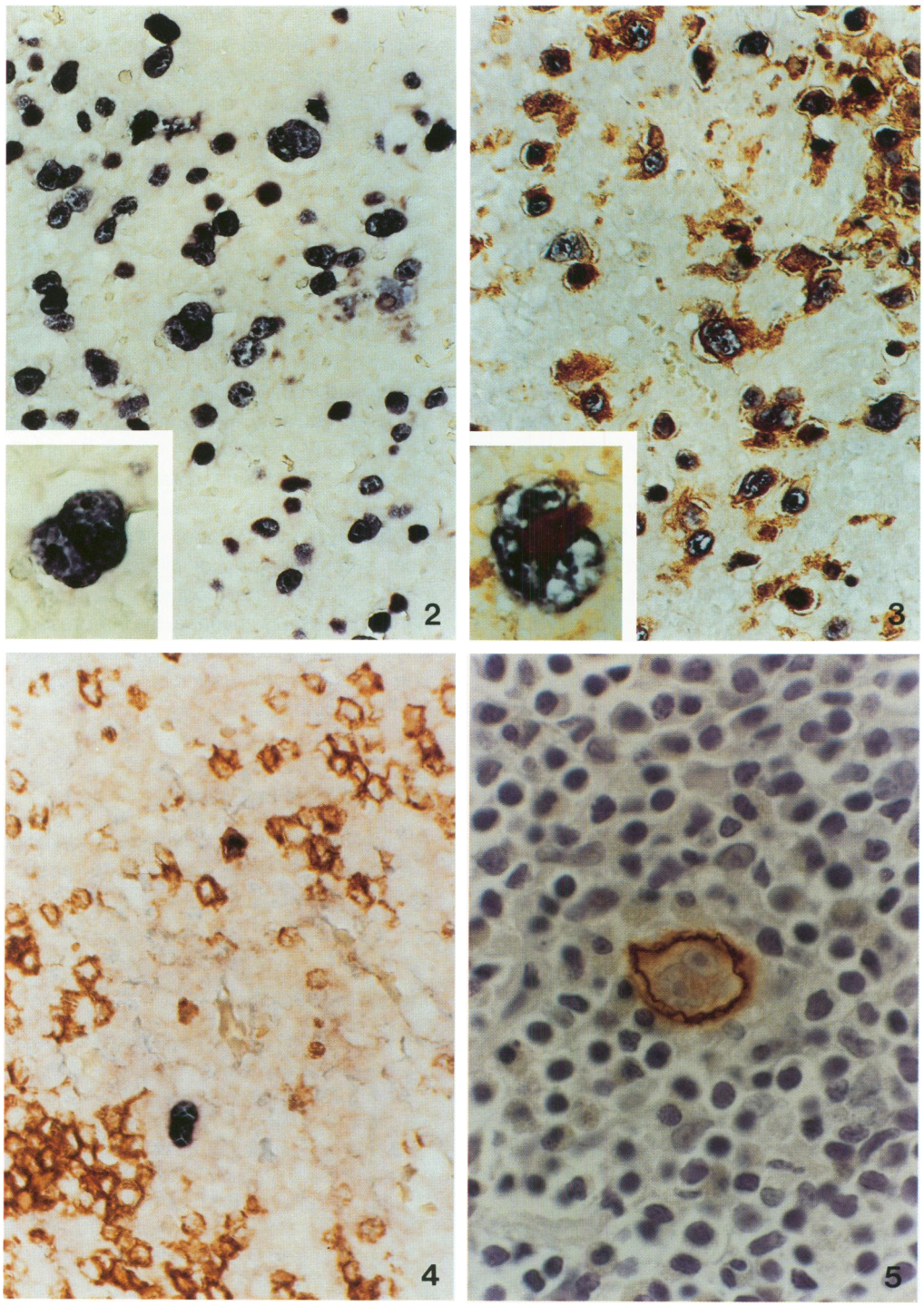

Figure 2. In situ *hybridization for EBV utilizing a biotinylated oligonucleotide probe directed against the EBER1 gene. All or virtually all of the Reed–Sternberg cells and variants show a blue-black labeling of their nuclei, indicating the presence of EBV RNA. Virtually all of these positive cells were labeled with CD15 (see Figure 3).*

Figure 3. *Double-labeling immunohistochemistry/*in situ *hybridization study utilizing LeuM1 (CD15) antibody and a biotinylated oligonucleotide probe directed against the EBER1 gene. The Reed–Sternberg cells and variants show co-labeling for CD15 (brown cytoplasmic and membranous staining) and EBV RNA (blue nuclear staining).*

Figure 4. *Double-labeling immunohistochemistry/*in situ *hybridization study utilizing L26 (CD20) antibody and a biotinylated oligonucleotide probe directed against the EBER1 gene. Note the rare small lymphocyte* **(top, center)** *co-labeling for CD20 (brown, membranous staining) and EBV RNA (blue nuclear staining), presumably representing a reactive, EBV-infected B lymphocyte. The Reed–Sternberg cell* **(bottom, center)** *expresses EBV RNA but does not label for CD20.*

Figure 5. *Immunohistochemical staining for LMP. Note membrane staining of Reed–Sternberg cell, whereas other cells are negative.*

EBV recrudescence as HIV-associated disease progresses, probably due to a progressive loss of T-cell-dependent viral surveillance, which in turn leads to an increased number of EBV-infected cells and increased copy number of EBV genomes within these cells.[24] As an example, EBV has previously been implicated in the oral lesion occurring in AIDS patients designated hairy leukoplakia and in approximately 50% of cases of AIDS-associated non-Hodgkin's lymphomas, particularly those involving the central nervous system.[9,25,26]

The presence of EBV in the neoplastic cells of Hodgkin's disease may be due to a prior infection of preneoplastic cells, infection of cells at the time of acquisition of the malignant phenotype, or a post-neoplastic superinfection. The observation that all or virtually all Reed–Sternberg cells and variants contain EBV in a given case favors either of the first two possibilities, although one cannot rule out the third possibility if EBV-infected tumor cells have a marked growth advantage over non-EBV infected tumor cells. The notion of a virus infecting an established tumor is not without precedent. We have previously reported an apparent EBV superinfection of an HIV-lymphoproliferative process that we have designated a polyclonal lymphoma, in which a monoclonal EBV-infected population arises as a direct result of the superinfection.[11] In support of this hypothesis, we had previously studied one case of HIV-associated Hodgkin's disease and found only a focal EBV infection of a subpopulation of the Reed–Sternberg cells in this case.[5]

The presence of EBV in Reed–Sternberg cells and variants could explain the apparent poor clinical behavior of AIDS-associated Hodgkin's disease, including the proclivity to present at high stages.[14] The presence of EBV in AIDS-associated non-Hodgkin's lymphomas is associated with significantly decreased survival.[27] EBV may alter both the proliferative capability and the biological properties of the Hodgkin's cells. For example, the open reading frame in EBV called BCRF1 mimics the activity of interleukin-10 (cytokine synthesis inhibitory factor)[28]; in the mouse, interleukin-10 or recombinant BCRF1 interferes with effector functions of T cells involved in delayed type hypersensitivity.[29] On the other hand, investigators have not observed a difference in stage or clinical behavior between EBV-associated Hodgkin's disease and EBV-negative Hodgkin's disease occurring in immunocompetent individuals.[30–32]

In addition to infection of the Hodgkin's cells, EBV RNA was also seen within smaller cells, identified to be primarily B cells by the double-labeling studies, in all cases in which the Reed–Sternberg cells were EBV-positive. These EBV-positive B cells, or a subset of these cells, may theoretically represent a population related to the neoplastic population, such as a precursor population, but we think that it is much more likely that these cells represent an expanded pool of EBV-positive non-neoplastic B cells, due to the immunosuppression present in these individuals. We have observed similar EBV-positive small cells in benign lymph node biopsies from HIV-infected patients and in biopsies of Hodgkin's disease in a non-HIV-infected population.[12]

In a previous study, 13 cases of nodular, lymphocyte predominance Hodgkin's disease were found to not contain EBV within the Reed–Sternberg variants found in this subtype, the L&H cells.[5] Similarly, we were not able to identify EBV RNA in the one case of HIV-associated nodular, lymphocyte predominance Hodgkin's disease. Because this subtype of Hodgkin's disease has a unique immunophenotypic profile and clinical course, different from other subtypes of Hodgkin's disease, it has been proposed that nodular, lymphocyte predominance Hodgkin's disease may represent an entity different from classical Hodgkin's disease.[33,34] Our findings represent additional evidence in support for the hypothesis.

Note Added in Proof

Recently, Audouin and colleagues identified LMP in the Reed-Sternberg cells of 16 cases of HIV-associated Hodgkin's disease.

References

1. Weiss LM, Strickler JG, Warnke RA, Purtilo DT, Sklar J: Epstein-Barr viral DNA in tissues of Hodgkin's disease. Am J Pathol 1987, 129:86–91
2. Staal SP, Ambinder R, Beschorner WE, Hayward GS, Mann R: A survey of Epstein-Barr virus DNA in lymphoid tissue: frequent detection in Hodgkin's disease. Am J Clin Pathol 1989, 91:1–5
3. Weiss LM, Movahed LA, Warnke RA, Sklar J: Detection of Epstein-Barr viral genomes in Reed-Sternberg cells of Hodgkin's disease. N Engl J Med 1989, 32: 502–506
4. Anagnostopoulos I, Herbst H, Niedobitek G, Stein H: Demonstration of monoclonal EBV genomes in Hodgkin's disease and Ki-1-positive anaplastic large cell lymphoma by combined Southern blot and *in situ* hybridization. Blood 1989, 74:810–816
5. Weiss LM, Chen Y-Y, Liu X-F, Shibata D: Epstein-Barr virus and Hodgkin's disease. A correlative *in situ* hybridization and polymerase chain reaction study. Am J Pathol 1991, 39:1259–1265

6. Brousset P, Chittal S, Schlaifer D et al: Detection of Epstein-Barr virus messenger RNA in Reed-Sternberg cells of Hodgkin's disease by *in situ* hybridization with biotinylated probes on specially processed modified acetone methyl benzoate xylene (ModAMeX) sections. Blood 1991, 77:1781–178
7. Herbst H, Dallenbach F, Hummel M et al: Epstein-Barr virus latent membrane protein expression in Hodgkin and Reed-Sternberg cells. Proc Natl Acad Sci USA 1991, 88:4766–4770
8. Pallesen G, Hamilton-Dutoit SJ, Rowe M, Young LS: Expression of Epstein-Barr virus latent gene products in tumour cells of Hodgkin's disease. Lancet 1991, 337:320–322
9. Meeker TC, Shiramizu B, Kaplan L et al: Evidence for molecular subtypes of HIV-associated lymphoma: division into peripheral monoclonal, polyclonal and central nervous system lymphoma. AIDS 1991, 5:669–674
10. Herndier BG: Surgical pathology of HIV associated lymphoproliferations. Cancer Surv 1991, 10:135–149
11. McGrath MS, Shiramizu B, Meeker TC, Kaplan LD, Herndier B: AIDS-associated polyclonal lymphoma: identification of a new HIV-associated disease process. J Acquir Immune Defic Syndr 1991, 4:408–415
12. Shibata D, Weiss LM, Nathwani BN, Brynes RK, Levine AM: Epstein-Barr virus in benign lymph node biopsies from individuals infected with the human immunodeficiency virus is associated with concurrent or subsequent development of non-Hodgkin's lymphoma. Blood 1991, 77:1527–1533
13. Knowles DM, Chamulak G, Subar et al: Clinicopathologic, immunophenotypic, and molecular genetic analysis of AIDS-associated lymphoid neoplasia. Pathology Annual, part 2. Edited by Rosen PP, Fechner RE, Appleton & Lange, Norwald, Connecticut, 1988, pp 33–67
14. Schoeppel SL, Hoppe RT, Dorfman RF et al: Hodgkin's disease associated with individuals at risk for the acquired immune deficiency syndrome. Ann Intern Med 1985, 102:68–70
15. Glickman JN, Howe JG, Steitz JA: Structural analyses of EBER1 and EBER2 ribonucleoprotein particles present in Epstein-Barr virus-infected cells. J Virol 1988, 62:902–911
16. Weiss LM, Movahed LA, Chen Y-Y et al: Detection of immunoglobulin light-chain mRNA in lymphoid tissues using a practical *in situ* hybridization method. Am J Pathol 1990, 137:979–988
17. Weiss LM, Chen Y-Y: Effects of different fixatives on the detection of nucleic acids in paraffin embedded tissues by *in situ* hybridization using oligonucleotide probes. J Histochem Cytochem 1991, 39:1237–1242
18. Sheibani K, Tubbs RR: Enzyme immunohistochemistry: technical aspects. Semin Diagn Pathol 1984, 1:235–250
19. Rowe BM, Evans HS, Young LS, Hennessy K, Kieff E, Rickinson AB: Monoclonal antibodies to the latent membrane protein of Epstein-Barr virus reveal heterogeneity of the protein and inducible expression in virus-transformed cells. J Gen Virol 1987, 68:1575–1586
20. Herbst H, Steinbrecher E, Niedobitek G et al.: distribution and phenotype of Epstein-Barr virus-harboring cells in Hodgkin's disease. Blood 1992, 80:484–491
21. Uccini S, Monard F, Stoppaccioro A et al: High frequency of Epstein-Barr virus genome detection in Hodgkin's disease of HIV-positive patients. Int J Cancer 1990, 46:581–585
22. Moran CA, Tuur S, Angritt P, Reid AH, O'Leary TJ: Epstein-Barr virus in Hodgkin's disease from patients with human immunodeficiency virus infection. Hum Pathol 1992, 5:85–88
23. Samoszuk M, Ravel J: Frequent detection of Epstein-Barr viral deoxyribonucleic acid and absence of cytomegalovirus deoxyribonucleic acid in Hodgkin's disease and acquired immunodeficiency syndrome-related Hodgkin's disease. Lab Invest 1991, 6:631–636
24. Birx DL, Redfield RR, Tosato G: Defective regulation of Epstein-Barr virus infection in patients with acquired immunodeficiency syndrome (AIDS) or AIDS related disorders. N Engl J Med 1986, 314:874–879
25. Greenspan, JS, Greenspan D, Lennette ET et al.: Replication of Epstein-Barr virus within the epithelial cells of oral 'hairy' leukoplakia, an AIDS-associated lesion. N Engl J Med 1985, 313:1565–1571
26. MacMahon EME, Glass JD, Hayward SD et al.: Epstein-Barr virus in AIDS-related primary central nervous system lymphoma. Lancet 1991, 338:969–973
27. Kaplan LD, Shiramizu B, Kahn JO et al: Molecular predictors of survival in HIV associated non-Hodgkin's lymphoma. VI. International Conference on AIDS. Florence, Italy, vol. 1, p 55
28. Hsu D-H, de Waal Malefyt R, Fiorentino DF et al.: Expression of interleukin-10 activity by Epstein-Barr virus protein BCRF1. Science 1991, 250:830–832
29. Moore KW, Vieira P, Fiorentino DF, Trounstine ML, Khan TA, Mosmann TR. Homology of cytokine synthesis inhibitory factor (IL-10) to the Epstein-Barr virus gene BCRF1. Science 1991, 248:1230–1234
30. Herbst H, Niedobitek G, Kneba M et al: High incidence of Epstein-Barr virus genomes in Hodgkin's disease. Am J Pathol 1990, 137:13–18
31. Jarrett RF, Gallagher A, Jones DB et al.: Detection of Epstein-Barr virus genomes in Hodgkin's disease: relation to age. J Clin Pathol 1991, 44:844–848
32. Vestlev PM, Pallesen G, Sandvej K, Hamilton-Dutoit SF, Bendtzen SM: Prognosis of Hodgkin's disease is not influenced by Epstein-Barr virus latent membrane antigen. Int J Cancer (letter) 1992, 51:1–3
33. Pinkus GS, Said JW: Hodgkin's disease, lymphocyte predominance type, nodular—further evidence for a B cell derivation. Am J Pathol 1988, 133:211–217
34. Regula DP, Hoppe RT, Weiss LM: Nodular and diffuse types of lymphocyte predominance Hodgkin's disease. N Engl J Med 1988, 318:214–219
35. Audouin J, Diebold J, Pallesen G. Frequent expression of Epstein-Barr virus latent membrane protein-1 in tumour cells of Hodgkin's disease in HIV-positive patients. J Pathol 1992, 167:381–384