Int J Clin Exp Pathol (2008) 1, 362-368
www.ijcep.com/IJCEP708011

*Original Article*

# Detection of Human Herpesvirus DNA in Kikuchi-Fujimoto Disease and Reactive Lymphoid Hyperplasia

S. David Hudnall[1], Tiansheng Chen[1], Samir Amr[2], Ken H. Young[3] and Kristin Henry[4]

*[1]Department of Pathology, University of Texas Medical Branch, Galveston, TX; [2]Pathology Services Division, Dhahran Health Center, Dhahran, Saudi Arabia; [3]Department of Pathology, University of Wisconsin School of Medicine and Public Health, Madison, WI and [4]Department of Histopathology, Charing Cross Hospital, Imperial College London, United Kingdom*

Received 23 Aug 2007; accepted with revision 27 Sept 2007; available online 28 Sept 2007

**Abstract**: Kikuchi-Fujimoto disease (KFD), or histiocytic necrotizing lymphadenitis, is a subacute inflammatory disorder most often seen in young women with clinicopathologic features suggestive of an infectious etiology. The most commonly suspected infectious agents in KFD are the human herpesviruses EBV, HHV6, HHV7 and HHV8. In order to identify herpesviruses in KFD, we have compared the frequency of detection of herpesvirus DNA with a recently developed real time PCR method, EBER in situ hybridization, and EBV latent membrane protein (LMP) immunostaining in 30 cases of KFD and 12 cases of reactive lymphoid hyperplasia (RLH). EBV DNA was commonly detected, while HSV2, CMV, HHV6, and HHV7 DNA were seldomly detected, and HSV1, VZV, and HHV8 DNA were not detected in KFD. EBV was also commonly detected in RLH. EBER-positive cells with apoptotic features were identified in necrotizing regions of many KFD cases, and LMP-positive cell debris was detected in one case. Viable EBER-positive cells were identified in four of twelve RLH cases, and rare LMP positivity detected in three cases. These data lend support to the notion that the necrotizing lesions in KFD may in some cases be due to a vigorous immune response to EBV-infected lymphoid cells.

**Key Words**: Herpesvirus, Kikuchi-Fujimoto disease, histiocytic necrotizing lymphadenitis, reactive lymphoid hyperplasia, Epstein-Barr virus, real time PCR

## Introduction

Kikuchi-Fujimoto disease (KFD), also known as histiocytic necrotizing lymphadenitis, is a self-limited febrile illness primarily of young women often accompanied by chills, myalgia, sore throat, skin rash, and cervical lymph-adenopathy. The distinctive lymph node histopathology is characterized by large patchy zones of necrosis with surrounding histiocytes, plasmacytoid monocytes, and immunoblasts. The necrotic zones contain numerous apoptotic bodies and nuclear dust with absence of neutrophils and eosinophils (**Figure 1**). Many histopathologic features of KFD are shared by systemic lupus lymphadenitis, but the lack of vasculitis and hematoxylin bodies favors KFD. The acute, self-limited, febrile nature of KFD has led to a strong suspicion that the disease is caused by an infectious agent. Suspected viral agents have included the herpesviruses Epstein-Barr virus (EBV) [1-8], human herpesvirus (HHV)6 [8, 9-11] and HHV8 [12]. On the other hand, other investigators have stressed that there is little evidence to support a herpesvirus etiology in KFD [13, 14]. George *et al* [12], while detecting EBV by PCR and in-situ hybridization in only 2 of 29 cases of KFD nevertheless concluded that the role of EBV in KFD remains an open question. The lack of agreement among various investigators regarding the putative infectious nature of this peculiar inflammatory condition continues to be problematic.

Previous studies have not examined KFD lymphoid tissue for all eight known herpesviruses and have not adequately controlled for herpesvirus positivity with reactive lymphoid tissues. Thus, in order to fully address the issue of herpesviral etiology of KFD we have examined a large number of cases of KFD collected from the United States,

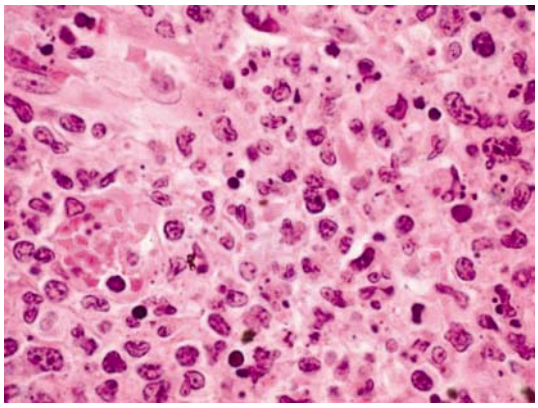

**Figure 1** Necrotic zone in KFD with plasmacytoid dendritic cells, macrophages, immunoblasts, and small apoptotic bodies (H&E, 400x).

**Table 1** Herpesvirus real time PCR primers and probes

| Targets | Oligonucleotide sequences |
|---|---|
| HSV1f | ATACCGACCACACCGACGA |
| HSV1r | ACAACTCCCTAACCCCTGCT |
| HSV1p | TET-AGGGGCCATTTTACGAGGAGGA-BHQ |
| HSV2f | TTCCCCCGTGGCTCAATATT |
| HSV2r | ACGCGCCGGGGCAGGTCT |
| HSV2p | ROX-TTATGCCTATCCCCGGTTGGACGA-BHQ |
| VZVf | GGCGGAACTTTCGTAACCAA |
| VZVr | CCCCATTAAACAGGTCAACAAAA |
| VZVp | Cy3-TCCAACCTGTTTTGCGGCGGC-BHQ |
| EBVf | CCAAGAAGGTGGCCCAGA |
| EBVer | CGGGTTGGAACCTCCTTG |
| EBVir | CCTGCCTCCATCACCCTG |
| EBVp | Fam-CCGCAGATGACCCAGGAGAAGGCC-BHQ |
| CMVf | TCGCGCCCGAAGAGG |
| CMVr | CGGCCGGATTGTGGATT |
| CMVp | Cy3-CACCGACGAGGATTCCGACAACG-BHQ |
| HHV6f | TCGAAATAAGCATTAATAGGCACACT |
| HHV6r | CGGAGTTAAGGCATTGGTTGA |
| HHV6p | TET-CCAAGCAGTTCCGTTTCTCTGAGCCA-BHQ |
| HHV7f | ATGTACCAATACGGTCCCACTTG |
| HHV7r | AGAGCTTGCGTTGTGCATGTT |
| HHV7p | ROX-AGCACGCACGGCAATAACTCTAGAAG-BHQ |
| HHV8f | TGCCCTGAGCCAGTTTGTC |
| HHV8r | AATAAACGCCGGGTCTGTACC |
| HHV8p | ROX- AACATGCCGCACACCGTCAG-BHQ |
| GAPDHf | ATTCCACCCATGGCAAATTC |
| GAPDHr | TGGGATTTCCATTGATGACAA G |
| GAPDHp | ROX-ATGGCACCGTCAAGGCTGAGAA-BHQ |

England, and Saudi Arabia for the presence of herpesvirus DNA with our recently developed real time PCR assay for detection of all eight human herpesviruses supplemented by EBER RNA in-situ hybridization and EBV LMP1 (latent membrane protein 1) immunoperoxidase staining, and compared these results with those obtained from control reactive lymphoid tissues.

## Materials and Methods

A total of 30 lymph nodes involved by KFD from the United Kingdom (cases 1-8), Saudi Arabia (cases 9-20), and the Wisconsin Pathology Network (cases 21-30) along with 12 reactive lymphoid tissues (including 2 cases of lupus lymphadenitis) were examined. Clinical information on the non-lupus reactive cases was non-contributory. DNA was extracted from three 10μm sections of each block according to manufacturer instructions (QIAamp DNA tissue kit, Qiagen) and quantified by ultraviolet spectrophotometry. Herpesvirus DNA was detected by a real time PCR technique with a set of 8 custom-designed herpesvirus-specific fluorescent TaqMan probes (**Table 1**). Given the relatively poor sensitivity of single step EBV real time PCR, pre-amplified EBNA1 (Epstein Barr nuclear antigen 1) PCR product rather than unamplified tissue DNA was used as starting material for real time PCR. In those cases in which no herpesvirus DNA was detected, GAPDH (glucose-6-phosphate dehydrogenase) PCR was performed to demonstrate presence of amplifiable genomic DNA.

Five micron tissue sections were prepared on positively-charged glass slides for immuno-histochemistry and EBER in-situ hybridization (ISH). After protease digestion, sections for ISH were subjected to room temperature hybridization with FITC-conjugated oligo-nucleotide probes to EBER1 (Epstein Barr early RNA 1) and EBER2 (Dako). After strenuous washing, sections were incubated with alkaline phosphatase conjugated anti-FITC antibody and the alkaline phosphatase substrate BCIP/NBT, followed by nuclear fast red counterstaining. After citrate buffer antigen retrieval, sections for immuno-histochemistry were incubated with primary monoclonal antibodies [anti-CD3, anti-CD20, anti-CD68, anti-EBV LMP1 (Dako)], rinsed with buffer, and subjected to antibody detection

**Table 2** Human herpesviruses in Kikuchi-Fujimoto disease

| Case | Origin | HSV1 | HSV2 | VZV | EBV | CMV | HHV6 | HHV7 | HHV8 | EBER | LMP |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | UK | - | - | - | + | - | - | - | - | + | - |
| 2 | UK | - | - | - | - | - | - | - | - | | |
| 3 | UK | - | - | - | + | - | - | - | - | - | - |
| 4 | UK | - | - | - | - | - | - | - | - | - | - |
| 5 | UK | - | - | - | + | - | - | - | - | + | |
| 6 | UK | - | - | - | + | - | - | - | - | + | |
| 7 | UK | - | - | - | + | - | - | - | - | - | - |
| 8 | UK | - | - | - | + | - | - | + | - | + | - |
| 9 | SA | - | - | - | + | - | - | - | - | - | |
| 10 | SA | - | - | - | - | - | - | - | - | - | - |
| 11 | SA | - | - | - | + | - | - | - | - | + | - |
| 12 | SA | - | - | - | - | - | - | - | - | + | - |
| 13 | SA | - | - | - | - | - | - | - | - | | |
| 14 | SA | - | - | - | + | - | - | - | - | | |
| 15 | SA | - | - | - | + | + | - | - | - | - | + |
| 16 | SA | - | - | - | - | - | - | - | - | + | - |
| 17 | SA | - | - | - | - | - | - | - | - | | |
| 18 | SA | - | - | - | - | - | - | - | - | | |
| 19 | SA | - | - | - | - | - | - | - | - | | |
| 20 | SA | - | - | - | - | - | - | - | - | | |
| 21 | USA | - | - | - | + | - | - | - | - | - | - |
| 22 | USA | - | - | - | + | - | - | - | - | - | - |
| 23 | USA | - | - | - | + | - | - | - | - | + | - |
| 24 | USA | - | - | - | + | - | - | - | - | + | |
| 25 | USA | - | - | - | - | - | + | + | - | - | - |
| 26 | USA | - | - | - | - | - | - | - | - | - | - |
| 27 | USA | - | - | - | + | - | + | + | - | - | - |
| 28 | USA | - | - | - | + | - | - | - | - | - | - |
| 29 | USA | - | + | - | + | - | - | - | - | - | - |
| 30 | USA | - | - | - | + | - | - | - | - | + | - |

HSV: herpes simplex virus; VZV: varicella zoster virus; EBV: Epstein-Barr virus; CMV: cytomegalovirus; HHV: human herpesvirus; EBER: Epstein-Barr early RNA; LMP: latent membrane protein.

with a highly sensitive immunoperoxidase technique (CSA II kit, Dako) using diaminobenzidine as chromagen, and counterstained with hematoxylin.

## Results

*Herpesvirus PCR*

Results of herpesvirus PCR testing of KFD are presented in **Table 2**. No herpesvirus DNA was detected in 11 cases (37%). EBV DNA was detected in 18 cases (60%), and was the only herpesvirus detected in 14 cases (47%). Other herpesviruses detected included HHV7 (3 cases), HHV6 (2 cases), CMV (1 case), and HSV2 (1 case). Herpesviruses HSV-1, VZV, and HHV-8 were not detected in any sample. While 78% of the KFD cases from the U.K. and U.S. were EBV positive, only 33% of the cases from

**Table 3** Human herpesviruses in lymph nodes with reactive lymphoid hyperplasia

| Case | Age/sex | Pattern | EBV | HHV6 | HHV7 | EBER | LMP |
|---|---|---|---|---|---|---|---|
| 1 | 34/F | follicular | + | - | + | - | - |
| 2 | 65/F | follicular | + | - | - | - | - |
| 3 | 57/F | follicular | + | + | + | - | - |
| 4 | 35/F | paracortical | + | + | + | - | - |
| 5 | 38/M | mixed | + | + | + | + | + |
| 6 | 21/F | follicular | + | - | - | + | - |
| 7 | 58/M | follicular | - | + | + | - | - |
| 8 | 37/F | mixed | + | - | - | + | + |
| 9 | 36/F | follicular | + | - | + | + | + |
| 10 | 41/F | follicular | + | - | + | - | - |
| 11 | unknown | lupus | + | + | - | - | - |
| 12 | unknown | lupus | + | - | + | - | - |

Saudi Arabia were EBV positive.

Controls included 10 lymph nodes with nonspecific reactive hyperplasia of which 9 were EBV positive, 4 HHV6 positive and 7 HHV7 positive (**Table 3**). The reactive hyperplasia cases included those with follicular, paracortical, and mixed follicular-paracortical patterns. Given the rarity with which they were detected in KFD, herpesviruses HSV1, HSV2, VZV, CMV, and HHV8 were not tested for in the reactive nodes. EBV, HHV6, and HHV7 were detected in 3 cases, EBV and HHV7 in 3 cases, HHV6 and HHV7 in 1 case, and EBV only in 3 cases. Two cases of lupus lymphadenitis with large areas of confluent necrosis closely mimicking the histopathology of KFD were also included as controls. One lupus case was positive for both EBV and HHV7 while another was positive for EBV and HHV6.

*Immunohistochemistry*

In order to better delineate the histopathology of the necrotizing lesions of KFD, formalin-fixed tissue sections of 4 KFD cases were stained for the T cell antigen CD3, the B cell antigen CD20, and the macrophage/ plasmacytoid dendritic cell antigen CD68 with a standard immunoperoxidase method. The more viable regions of the lymph nodes were characterized by numerous CD20-positive B cell rich primary and secondary reactive lymphoid follicles often surrounded by prominent B cell rich mantle zones. In contrast, most cells within the necrotizing foci were large CD68-positive phagocytic macrophages and plasmacytoid dendritic cells (**Figure 2A**), with scattered medium to large CD3-positive T cells (**Figure 2B**), and relatively few scattered medium to large CD20-positive B cells (**Figure 2C**). Some of the B cells within the necrotizing foci exhibited morphologic features of large Reed-Sternberg-like immunoblasts. While the small apoptotic cells failed to stain with CD20 and CD68, some apoptotic cells appeared to stain with the T cell marker CD3.

*EBER In-Situ Hybridization*

Ten of 23 KFD cases for which tissue sections were available contained EBER positive cells, eight of which were also positive by EBV PCR (**Table 2**). Positivity was confined to medium-sized lymphoid cells and small apoptotic cells located within the necrotic zones (**Figures 2D** and **2E**). No large EBER positive immunoblasts or histiocytes were identified, and no positive cells were found in adjacent reactive lymphoid tissue. No EBER ISH positive cells were detected in the 2 cases of lupus lymphadenitis. Three of 12 RLH control cases contained scattered viable-appearing EBER positive lymphocytes (**Table 3**, **Figure 2G**). In

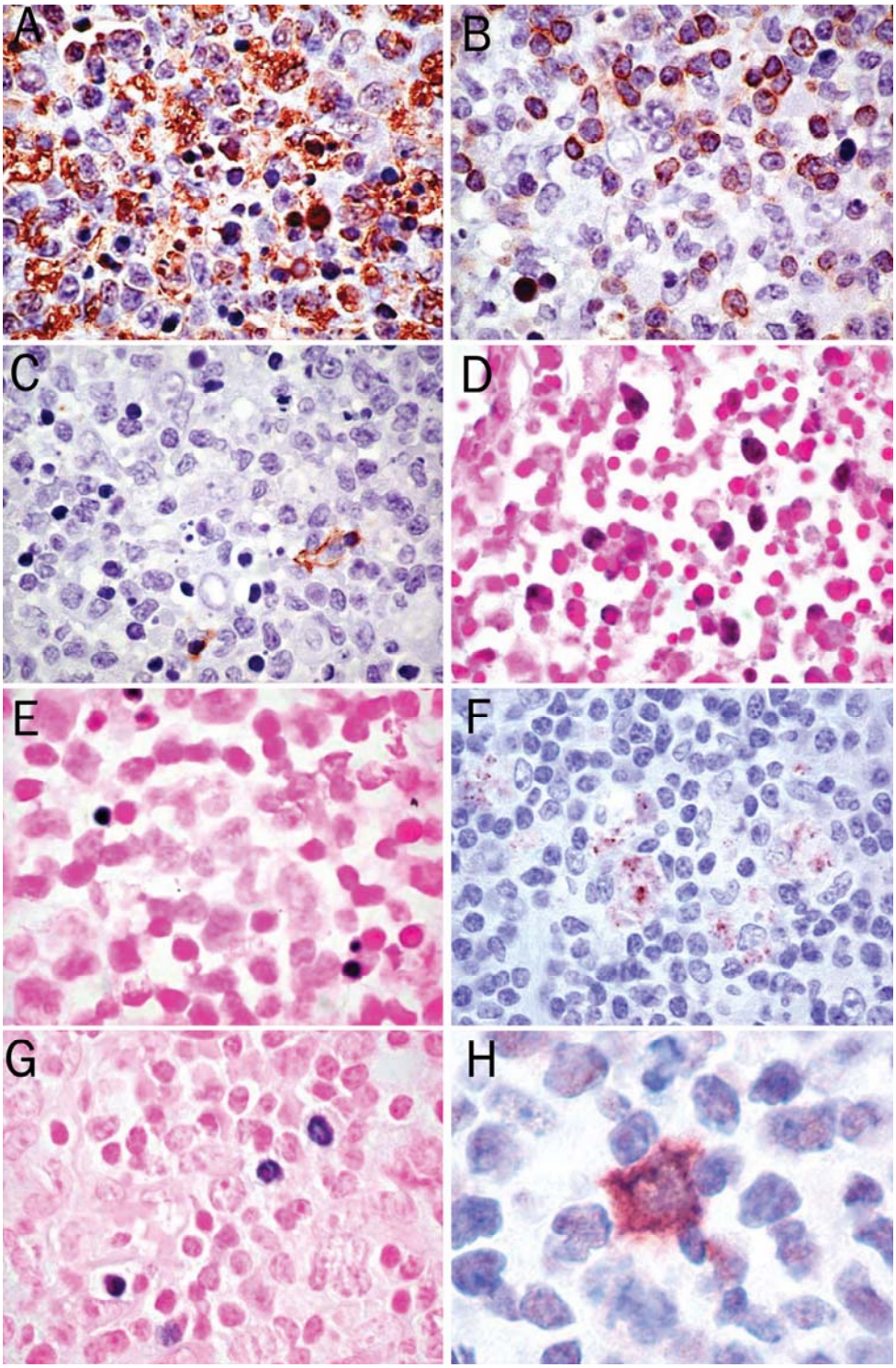


**Figure 2** **A**. Necrotic zone with numerous CD68 positive plasmacytoid dendritic cells and macrophages (CD68 immunoperoxidase, 600x). **B**. Necrotic zone with numerous CD3 positive T cells (CD3 immunoperoxidase, 600x). **C**. Necrotic zone with few CD20 positive B cells (CD20 immunoperoxidase, 600x). **D**. Necrotic zone with viable EBER positive cells (EBER in-situ hybridization, 600x). **E**. Necrotic zone with apoptotic EBER positive cells (EBER in-situ hybridization, 1000x). **F**. EBV LMP1 positivity of phagocytic debris within viable histiocytes in KFD (LMP1 immunoperoxidase, 600x). **G**. Viable EBER positive cells in reactive lymphoid hyperplasia (EBER in-situ hybridization, 600x). **H**. A rare viable LMP1 positive lymphoid cell in reactive lymphoid hyperplasia (LMP1 immunoperoxidase, 1000x)

contrast to KFD, no EBER positive apoptotic cells were identified in any RLH case.

*EBV LMP1 Immunoperoxidase*

EBV LMP1 immunoperoxidase staining was detected in only 1 of 19 KFD cases for which tissue sections were available. The positive case was EBV PCR positive but EBER ISH negative. LMP positivity was seen as granular cytoplasmic positivity within scattered phagocytic histiocytes, presumably derived from remnants of phagocytized EBV-positive lymphoid cells (**Figure 2F**). Very rare viable-appearing LMP1 positive lymphocytes were identified in 3 of the RLH control cases (**Table 3**, **Figure 2H**). No LMP positive phagocytic debris was identified in any case of RLH. These results indicate that in contrast to EBV-positive Hodgkin lymphoma, only rare EBER-positive cells in RLH express detectable levels of LMP1 protein.

## Discussion

In order to explore the role of herpesvirus infection in Kikuchi-Fujimoto disease (KFD), we have examined 30 cases of KFD from the U.S., U.K., and Saudi Arabia for the presence of PCR-amplifiable herpesvirus DNA using formalin-fixed paraffin-embedded lymph node tissue, and compared these data with those obtained from 12 cases of reactive lymphoid hyperplasia from the U.S., including 2 cases of necrotizing lupus lymphadenitis.

Since HSV1, VZV, and HHV8 DNA was not detected, and HSV2, CMV, HHV6, and HHV7 DNA was only occasionally detected in the KFD lymph nodes (with HHV6 and HHV7 more often detected in RLH), we conclude that these herpesviruses are highly unlikely to be involved in the pathogenesis of KFD. In contrast, EBV DNA was detected in most of the KFD samples. However, since EBV DNA was also detected in most of the reactive lymph nodes, on the basis of these PCR results alone we can draw no conclusions regarding the role of EBV in KFD.

It is interesting to note that EBV DNA positivity was significantly greater in KFD cases from the U.S. (80%) and U.K. (75%) as compared with cases from Saudi Arabia (33%) ($p < 0.001$, Chi square test). While this result might suggest that EBV is more commonly associated with KFD in the U.S. and U.K than Saudi Arabia, we cannot exclude the possibility that this result merely reflects a geographical difference in the rate of lymph node EBV positivity in the general population.

Results of EBER ISH and EBV LMP1 immunostaining of KFD were quite interesting. In KFD nodes, EBER positivity was confined to small apoptotic and medium-sized lymphoid cells within the necrotic zones. EBER positivity was also identified in rare scattered medium-sized lymphoid cells in 4 of 12 reactive nodes. In contrast, no apoptotic EBER-positive cells were identified in the reactive nodes. LMP1 immunoreactivity was seen in one KFD case in an unusual histologic pattern, i.e. punctuate cytoplasmic positivity of histiocytes within the necrotic foci. In our experience, this pattern of LMP positivity (real or artifactual) has not previously been reported in reactive or neoplastic lymph nodes. We suspect that this pattern of immunoreactivity may be due to histiocytic phagocytosis of EBV-positive cells. This unusual pattern of LMP1 immuno-reactivity was not seen in any RLH case in the current study. In contrast, only rare viable medium-sized lymphoid cells with diffuse cytoplasmic LMP1 positivity were seen in RLH. The presence of EBV infected cells with apoptotic cytologic features concentrated within necrotic zones of KFD suggests that the necrotizing immune reaction seen in KFD may at least in some cases be directed against EBV-infected cells.

Discovery of infectious agents in KFD may be hampered by the fact that many lymph node biopsies are obtained relatively late in the course of the illness after many putatively infected lymphoid cells have undergone apoptotic cell death [15]. At this late stage of the illness it is likely that very little if any infectious agent DNA or protein remains. Thus, the difficulty in consistently detecting infectious agent DNA or protein in these biopsies might be expected, leading to both positive and negative results depending upon the quantity of high quality amplifiable viral/bacterial DNA or immunoreactive protein in the tissue. The present findings suggest that the necrotic reaction of late stage KFD may at least in part be due to apoptosis and phagocytosis of virus-infected lymphoid cells. Evidence supporting this hypothesis may come from careful examination of lymph nodes from

patients in the early pre-necrotic stage of KFD for the presence of viable virus-infected cells, serologic studies to detect an immune response to active virus infection, and viral load assays of peripheral blood.

*Please address all correspondences to S. David Hudnall, MD, Department of Pathology, University of Texas Medical Branch, 301 University Blvd., Galveston, Texas 77555-0741. Email: shudnall@utmb.edu*

## References

[1] Anagnostopoulos I, Hummel M, Korbjuhn P, Papadaki T, Anagnostou D and Stein H. Epstein-Barr virus in Kikuchi-Fujimoto disease. *Lancet* 1993;341:893.

[2] Hollingsworth HC, Peiper SC, Weiss LM, Raffeld M and Jaffe ES. An investigation of the viral pathogenesis of Kikuchi-Fujimoto disease. Lack of evidence for Epstein-Barr virus or human herpesvirus type 6 as the causative agents. *Arch Pathol Lab Med* 1994;118:134-140.

[3] Cho KJ, Lee SS and Khang SK. Histiocytic necrotizing lymphadenitis. A clinicopathologic study of 45 cases with in situ hybridization for Epstein-Barr virus and hepatitis B virus. *J Korean Med Sci* 1996;11:409-414.

[4] Yen-Moore A, Fearneyhough P, Raimer S and Hudnall SD. EBV-associated Kikuchi's histiocytic necrotizing lymphadenitis with cutaneous manifestations. *J Am Acad Dermatol* 1997;36:342-346.

[5] Chiu CF, Chow KC, Lin TY, Tsai MH, Shih CM and Chen LM. Virus infection in patients with histiocytic necrotizing lymphadenitis in Taiwan. Detection of Epstein-Barr virus, type I human T-cell lymphotropic virus, and parvovirus B19. *Am J Clin Pathol* 2000;113:774-781.

[6] Stephan JL, Jeannoel P, Chanoz J and Gentil-Perret A. Epstein-Barr virus-associated Kikuchi disease in two children. *J Pediatr Hematol Oncol* 2001;23:240-243.

[7] Carlson JA, Perlmutter A, Tobin E, Richardson D and Rohwedder A. Adverse Antibiotic-Induced Eruptions Associated With Epstein Barr Virus Infection and Showing Kikuchi-Fujimoto Disease-Like Histology. *Am J Dermatopathol* 2006;28:48-55.

[8] Maeda N, Yamashita Y, Kimura H, Hara S and Mori N. Quantitative analysis of herpesvirus load in the lymph nodes of patients with histiocytic necrotizing lymphadenitis using a real-time PCR assay. *Diagn Mol Pathol* 2006; 15:49-55.

[9] Hoffmann A, Kirn E, Kuerten A, Sander C, Krueger GR and Ablashi DV. Active human herpesvirus-6 (HHV-6) infection associated with Kikuchi-Fujimoto disease and systemic lupus erythematosus (SLE). *In Vivo* 1991;5:265-269.

[10] Krueger GR, Huetter ML, Rojo J, Romero M and Cruz-Ortiz H. Human herpesviruses HHV-4 (EBV) and HHV-6 in Hodgkin's and Kikuchi's diseases and their relation to proliferation and apoptosis. *Anticancer Res* 2001;21:2155-2161.

[11] Dominguez DC, Torres ML and Antony S. Is human herpesvirus 6 linked to Kikuchi-Fujimoto disease? The importance of consistent molecular and serologic analysis. *Clin Immunol* 2005;114:27-29.

[12] Huh J, Kang GH, Gong G, Kim SS, Ro JY and Kim CW. Kaposi's sarcoma-associated herpesvirus in Kikuchi's disease. *Hum Pathol* 1998; 29:1091-1096.

[13] Martinez-Vazquez C, Potel C, Angulo M, Gonzalez-Carrero J, Alvarez M, Tenorio A, Cid D and Oliveira I. Nosocomial Kikuchi's disease--a search for herpesvirus sequences in lymph node tissues using PCR. *Infection* 2001;29: 143-147.

[14] George TI, Jones CD, Zehnder JL, Warnke RA and Dorfman RF. Lack of human herpesvirus 8 and Epstein-Barr virus in Kikuchi's histiocytic necrotizing lymphadenitis. *Hum Pathol* 2003; 34:130-135.

[15] Hudnall SD. Kikuchi-Fujimoto disease. Is Epstein-Barr virus the culprit? *Am J Clin Pathol* 2000;113:761-764.