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Co-expression of Epstein-Barr virus latent membrane protein and vimentin in "aggressive" histological subtypes of Hodgkin's disease

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Summary. The presence of Epstein-Barr virus (EBV) genome in Hodgkin's and Reed-Sternberg (HRS) cells, as detected using in situ hybridization (ISH) with biotinylated *Bam*HI "V" probes, along with the expression of EBV-encoded latent membrane protein (LMP) and vimentin was examined in paraffin-embedded sections of 39 immunomorphologically characterized cases of Hodgkin's disease (HD). ISH demonstrated EBV in HRS cells in 15 of 39 cases, whereas LMP expression was detected in 11 of 39 cases, only in the presence of EBV genome detection. With the exception of 1 case, in which HRS cells expressed B-cell-associated antigens, the LMP-positive cases included specimens in which HRS cells were of non-B, non-T phenotype. LMP expression showed a stronger association with lymphocyte depletion (LD) (3/3) and mixed cellularity (MC) (6/11) than with lymphocyte predominance (0/5) or nodular sclerosis (2/20) subtypes. Vimentin expression on HRS cells was found in all the LMP-expressing cases and only in a fraction (13/28) of LMP-negative cases. This study supports the view that HD represents a heterogeneous group of diseases also in terms of EBV association, LMP expression being strongly related to the "aggressive" LD and MC histological subtypes. In light of the supposed interactions between vimentin and LMP, their co-expression on HRS cells, as detected in this study, provides further evidence for a significant role of EBV in the development of a proportion of HD cases.

Key words: Hodgkin's disease – Epstein-Barr virus – Latent membrane protein – Vimentin – Messenger RNA

Introduction

Hodgkin's disease (HD) represents a rather heterogeneous group of disorders not only in terms of clinical behaviour (Colby et al. 1981; MacLennan et al. 1989), morphology (Anastasi and Variakojis 1988; Colby et al. 1981; MacLennan et al. 1989), and immunophenotypes (Agnarsson and Kadin 1989; Angel et al. 1987; Diebold and Audouin 1989; Falini et al. 1987; Kadin et al. 1988), but also in terms of viral association. Recent studies with Southern blot analysis (Anagnostopoulos et al. 1989; Boiocchi et al. 1989; Herbst et al. 1990; Masih et al. 1991; Staal et al. 1989; Weiss et al. 1987), polymerase chain reaction (PCR) (Herbst et al. 1990, 1991), and in situ hybridization (ISH) (Anagnostopoulos et al. 1989; Brousset et al. 1991; Weiss et al. 1987, 1989; Wu et al. 1990) have demonstrated the presence of Epstein-Barr virus (EBV) genomes, in clonal and episomal form (Anagnostopoulos et al. 1989; Boiocchi et al. 1989; Masih et al. 1991; Weiss et al. 1989), in HD; however, the percentage of positive cases ranged from 20% to 70%, adding to its heterogeneity but supporting the concept of EBV as an aetiological agent in a proportion of HD cases. Other studies utilizing the PCR (Boiocchi et al. 1992; Weiss et al. 1991) or the analysis of EBV-latent membrane protein (LMP) (Pallesen et al. 1991) have indicated the presence of EBV genomes in most cases of mixed cellularity subtype of HD, thus raising the view that this subtype may selectively be an EBV-associated disease.

EBV-encoded LMP, which may be demonstrated effectively in conventional paraffin-embedded material (Delsol et al. 1992; Murray et al. 1992), is one of the few proteins encoded by the virus in latent infection associated with B-lymphocyte immortalization (Hennessy et al. 1984). LMP can induce many B-lymphocyte phenotypic characteristics which are evident in EBV infection, including increased surface expression of activation markers, and increased vimentin expression (Birkenbach et al. 1989; Wang et al. 1988). Interestingly, two independent immunohistological studies have demonstrated that vimentin expression on Hodgkin's and Reed-Sternberg (HRS) cells could be found only in a fraction of HD cases investigated (Carbone et al. 1990b; Tamaru et al. 1990).

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The current study examines the presence of the EBV genomic material in HRS cells, as detected using ISH with biotinylated (non-isotopic) *Bam*HI "V" probes, along with the expression of both EBV-encoded LMP and vimentin in paraffin-embedded sections of immunomorphologically characterized HD cases. These findings are correlated with the histological subtypes and the phenotypes of HRS cells to ascertain whether the virus is associated with specific subgroups of the disease.

Materials and methods

Morphological, immunohistological, and ISH studies were carried out on a series of 39 HD cases occurring in adult patients. Cases were retrieved non-randomly from the files of the Division of Pathology, Centro di Riferimento Oncologico. HD cases were selected in order to obtain a series representative of all major histological subtypes. According to the Rye modification of the Lukes and Butler classification (Lukes et al. 1966) histological subtypes were: 5 lymphocyte predominance (LP), 11 mixed cellularity (MC), 3 lymphocyte depletion (LD) and 20 nodular sclerosis (NS) (Table 1).

In all cases monoclonal antibodies (mAbs) suitable for paraffin tissues – leucocyte common antigen (LCA; CD45), BerH2 (CD30), LeuM1 (CD15), epithelial membrane antigen (EMA), vimentin, LN1 (CDw75), LN2 (CD74), LN3, L26 (CD20), MB1/MT2 (CD45R), MB2, CD3, UCHL1 (CD45RO), Leu22/MT1 (CD43), KP1 (CD68), anti-cytokeratin (MNF116) – were used as previously reported (Carbone et al. 1990a). In addition, for BerH2, vimentin, and CD3 antibodies the avidin-biotin-peroxidase complex (ABC) method was employed by using the ABC Elite kit (Vector, Burlingame, Calif., USA). The immunoreactivity along with the source of all these commercially available antibodies have been reported in separate papers (Carbone et al. 1990a, 1990c, 1992). A case was considered to show positive staining of unequivocal staining for that antigen was demonstrated on at least several HRS cells, according to Agnarsson and Kadin (1989).

Anti-LMP was available as a pool of four anti-LMP mAbs (CS.1–4; Rowe et al. 1987; Dakopatts A/S, Glostrup, Denmark). Immunostaining was performed on Bouin-fixed paraffin-embedded tissue sections by the alkaline phosphatase-antialkaline phosphatase method (Cordell et al. 1984). Positive controls were included in all test runs and consisted of sections of LMP expressing EBV-positive cell lines. Negative controls consisted of consecutive test sections in which primary antibody was replaced with non-immune serum of the same IgG subclass (Dakopatts).

For ISH the probe for EBV consisted of 1 μg/ml biotinylated EBV DNA prepared from the 3.1 kb *Bam*HI "V" (internal repeat 1) fragment of the EBV genome (cloned into pUC18 a 2.68 kb vector DNA) (Enzo Diagnostics, New York, USA) in hybridization cocktail (50% formamide, 5 × SSC, 1 × Denhardt solution, 10% dextran sulphate, 100 μg/ml salmon testes (DNA). In addition, EBV probe from a commercial kit (Enzo) was used in several cases.

ISH studies were performed on Bouin-fixed paraffin-embedded tissue sections using a modified procedure based on previously described methods (Bashir et al. 1989; Brigati et al. 1983; Lewis et al. 1987). Sections (2–4 μm), were floated in a distilled water both, taken up on glass slides pretreated with Vectabond reagent (Vector), baked overnight and then stored in a box at room temperature. The day of the test the sections were dewaxed, hydrated, and treated with 0.2 M hydrochloric acid (for 10 min). Proteolytic digestion of tissues was achieved with proteinase K (Sigma, St. Louis, Mo., USA) for 20 min at 37° C) at concentrations ranging from 2 to 40 μg/ml in TRIS-buffered saline (TBS); the extent of digestion required was determined empirically for each case. Sections were then incubated in TRIS-glycine buffer (Sigma) for 20 min, and stored in 2 × SSC (0.3 M sodium chloride, 0.03 M sodium citrate, pH 7) (Sigma) 50% formamide (Oncor, Gaithersburg, Md., USA) until hybridization (Bashir et al. 1989).

Table 1. Presence of Epstein-Barr (EBV) genomic material and immunoglobulin light chain messenger RNA in Hodgkin's and Reed-Sternberg cells (as detected using non-isotopic in situ hybridization) and expression of EBV-encoded latent membrane protein in 39 immunomorphologically characterized Hodgkin-s disease cases

Case no.	Histological subtype	Immunophenotype[a]	mRNA[a] (κ, λ)	Vimentin[a]	EBV-DNA	LMP
1	LP	Non-B, non-T	ND	−	−	−
2	LP	B-	−	−	−	−
3	LP	B-	ND	−	−	−
4	LP	B-	ND	−	−	−
5	LP	B-	−	−	−	−
6	NS	Non-B, non-T	−	+	+	+
7	NS	Non-B, non-T	−	+	+	−
8	NS	Non-B, non-T	−	+/−	+	+
9	NS	Non-B, non-T	−	+/−	+	−
10	NS	Non-B, non-T	ND	+/−	+/−	−
11	NS	Non-B, non-T	−	+	−	−
12	NS	Non-B, non-T	ND	+	−	−
13	NS	T-	−	+	−	−
14	NS	Non-B, non-T	ND	+	−	−
15	NS	Non-B, non-T	−	+	−	−
16	NS	B- and T-	−	+/−	−	−
17	NS	B- and T-	−	+/−	−	−
18	NS	Non-B, non-T	ND	+/−	−	−
19	NS	Non-B, non-T	ND	−	−	−
20	NS	B-	−	−	−	−
21	NS	Non-B, non-T	ND	−	−	−
22	NS	Non-B, non-T	−	−	−	−
23	NS	Non-B, non-T	−	−	−	−
24	NS	Non-B, non-T	ND	−	−	−
25	NS	Non-B, non-T	ND	−	−	−
26	MC	Non-B, non-T	−	+	+	+
27	MC	B-	−	+	+	+
28	MC	Non-B, non-T	−	+/−	+	+
29	MC	Non-B, non-T	−	+	−/+	−/+
30	MC	Non-B, non-T	−	+	−/+	−/+
31	MC	Non-B, non-T	−	+/−	−/+	−
32	MC	Non-B, non-T	−	+/−	−/+	+
33	MC	Non-B, non-T	−	+/−	−	−
34	MC	Non-B, non-T	ND	−	−	−
35	MC	Non-B, non-T	ND	−	−	−
36	MC	Non-B, non-T	ND	−	−	−
37	LD	Non-B, non-T	−	+	+	+
38	LD	Non-B, non-T	−	+	+	+
39	LD	Non-B, non-T	−	+	+	+

[a] Hodgkin's and Reed-Sternberg cells are concerned

+, Many positive cells; +/−, several positive cells; −/+, few positive cells; −, negative; mRNA, messenger RNA; LMP, latent membrane protein; LP, lymphocyte predominance; NS, nodular sclerosis; MC, mixed cellularity; LD, lymphocyte depletion; ND, not done

Probe DNA and tissue DNA were denatured simultaneously (Brigati 1983) by heating the slides in a convection oven, for 15 min, at a temperature of approximately 95° C; after overnight hybridization at 42° C (Lewis et al. 1987) coverslips were removed and the sections placed first in a solution of 2 × SSC in 50% formamide for 30 min at 42° C and then in 2 × SSC at 42° C for another 30 min; they were washed three times in 1 × SSC for 10 min each at room temperature with shaking, once in 0.1 × SSC at 42° C for 10 min, and finally placed in TBS pH 7.6 for 5 min. Hybridized sites were revealed using a kit from Dako (K600) (Dakopatts)

as described by the manufacturer. This system consists of sequential application of streptavidin and biotinylated alkaline phosphatase followed by development of alkaline phosphatase by using 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium. Colour development took from 30 min to 2.5 h and was checked every 30 min to monitor the progress of the reaction. Finally, sections were counterstained with nuclear fast red, dehydrated through graded alcohols, immersed in xylene and mounted with permanent mounting medium (Eukitt).

Cells were scored positive for viral DNA if they showed deposition of grains in excess. Controls for the ISH assay consisted of slides containing both a negative control well of fixed Ramos cells and positive control well of fixed B95.8 cells (purchased from Enzo). Furthermore, B95.8 and Raji cells (gift of Drs. M. Boiocchi and V. De Re) centrifuged into a pellet were Bouin-fixed, paraffin-embedded and treated according to a standard regimen used in the laboratory. The sectioned material was hybridized as described above. An additional negative control consisted of omission of the biotinylated probe from the hybridization cocktail.

In all cases Southern blot hybridization and PCR analyses for the detection of EBV genome were performed as previously described (Boiocchi et al. 1990, 1992). Data from these analyses were used only to confirm the respective findings and are not included in this report.

The ISH technique was used to detect immunoglobulin light chain messenger RNA (mRNA) in representative cases, including those in which HRS cells expressed B-cell-associated antigens. Kappa (κ) and lambda (λ) light chain mRNA could be detected in Bouin-fixed, paraffin-embedded sections, by using a commercial kit (Dako A/S no. K003) under conditions recommended by the supplier. Positive controls were provided by examining sections from a case of plasmacytoma, and of reactive tonsils. Negative controls were provided by incubating sections in hybridization solution to which no probe had been added.

Results

Almost all cases of HD were found to express the CD15 antigen (37/39, 94.9%). With the exception of the LP subtype, BerH2 (CD30) was expressed in nearly all cases (33/34, 97%). The five cases of the LP subtype expressed the following characteristic profile: $CD15^+$, $CD30^-$, $CD45^+$, $EMA^{-+/-}$.

In 6 cases staining of several or the majority of HRS cells with antibodies that recognize antigens on B-lymphocytes (CDw75, CD45R, CD20) was seen. In 1 case of the NS subtype a presumptive T-phenotype could be ascribed on the basis of the presence of CD43 and CD3 immunoreactivity on HRS cells, whereas in 2 other cases of the NS subtype HRS cells showed co-expression of B- and T-cell-associated antigens. The remaining 30 cases could not be categorized using the antibodies available (Table 1).

None of the HRS cells in any of the cases of HD studied by the use of biotinylated oligonucleotide probes specific for the constant regions of κ and λ light chain mRNA in ISH experiments showed positive staining with either probe.

In 24 of the 39 cases (61.5%) HRS cells were found to express vimentin (Fig. 1, Table 1); however, in the MC+LD subtypes vimentin expression was found in 11 of 14 cases (78.5%).

ISH demonstrated EBV in HRS cells in 15 of 39 cases (38.4%), as indicated in Table 1. Three of 3 LD were positive, as were 7 of 11 MC and 5 of 20 NS. The intensity of the hybridization signal was usually low, but it varied between cases. The EBV signal was nuclear (Fig. 2); the number of positive HRS cells varied between cases.

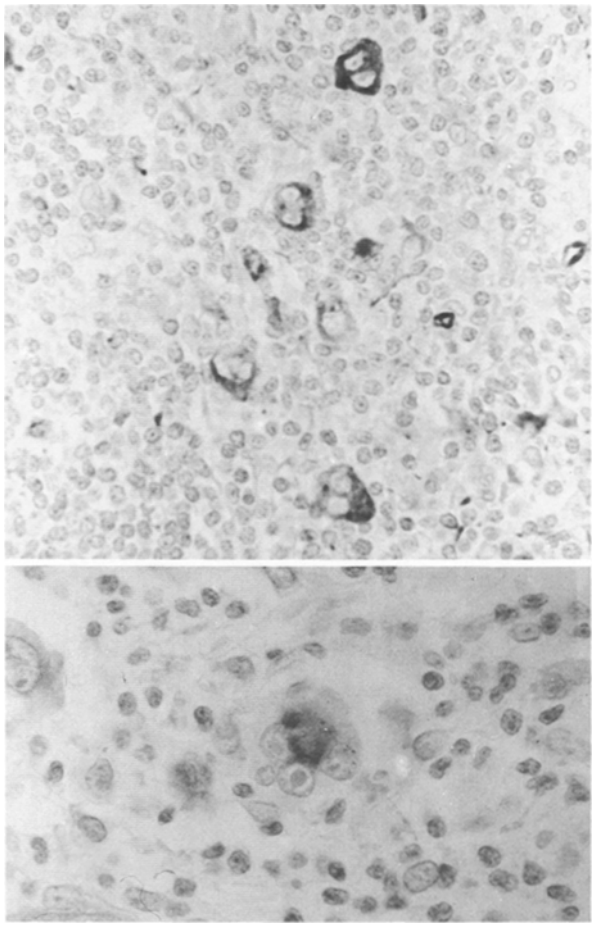

Fig. 1. Hodgkin's and Reed-Sternberg (RS) cells show strong and diffuse cytoplasmic positivity for vimentin (*top*). A giant multinucleated RS cell exhibiting paranuclear cytoplasmic positivity is also seen (*bottom*). Bouin-fixed, paraffin-embedded section of lymph node with mixed cellularity Hodgkin's disease (case 30); avidin biotin complex Elite; haematoxylin counterstain; ×400 (*top*), ×520 (*bottom*)

LMP expression was detected in 11 of 39 cases (28.2%) of HD examined; it was found only in the presence of EBV genomic material detection (Fig. 3). Positivity was found both on the cell surface and within the cytoplasm of HRS cells. In positive cases, the percentage of LMP expressing HRS cells ranged from 5% to 75%. Where reactive or foamy histiocytes were seen, they contained weakly stainable LMP. Small lymphocytes in the background were negative.

As shown in Table 2, HRS cells in all the cases of the LP subtype did not stain. Conversely, in all the LD cases tested LMP-positive HRS cells were detectable at low-power examination because of the strong cytoplasmic and membrane staining in most instances. Of the MC group, LMP was expressed by 6 of 7 cases which showed the presence of EBV nucleic acids. Of the NS group, LMP was expressed by 2 of 5 cases which showed

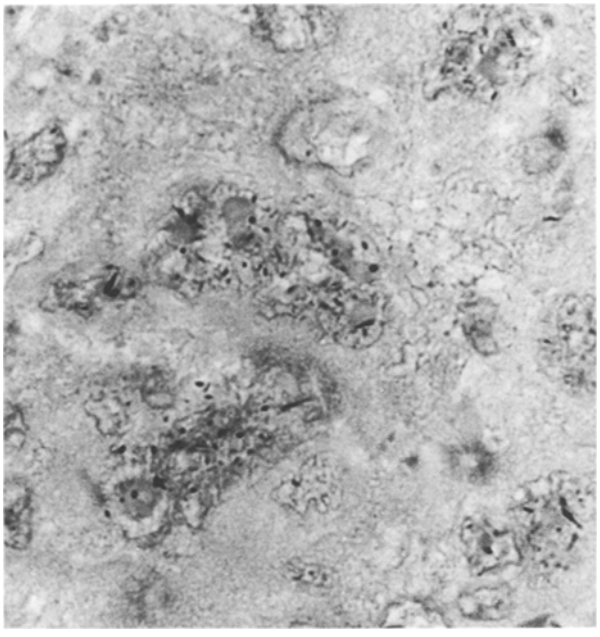

Fig. 2. Nuclear localization of Epstein-Barr virus (EBV) nucleic acid hybridization signal in diagnostic RS cells. Bouin-fixed, paraffin-embedded section of lymph node with nodular sclerosis Hodgkin's disease (case 6); biotinylated EBV probe; nuclear fast red counterstain; ×1000 oil immersion

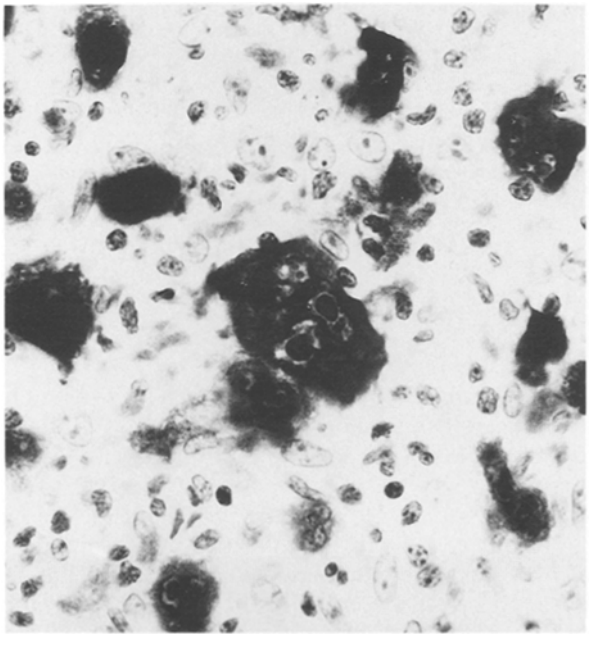

Fig. 3. Immunohistological detection of EBV-encoded latent membrane protein (LMP) expression confined to Hodgkin's and RS cells. Bouin-fixed, paraffin-embedded section of lymph node with nodular sclerosis Hodgkin's disease (same case as Fig. 2); alkaline phosphatase-anti-alkaline phosphatase (APAAP); haematoxylin counterstain; ×800

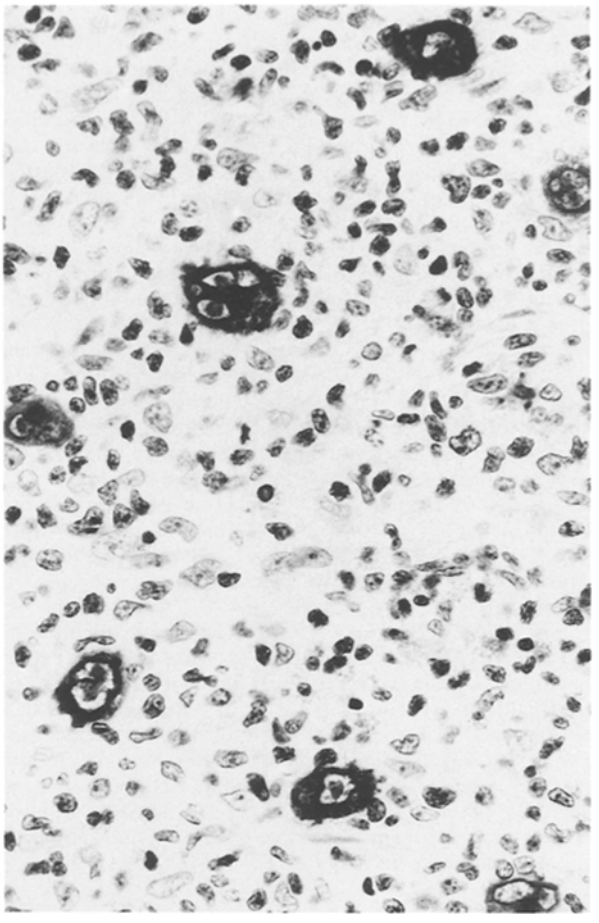

Fig. 4. A paraffin section from a specimen of mixed cellularity Hodgkin's disease exhibiting Hodgkin's and RS cells with strong staining for LMP. Bouin-fixed, paraffin-embedded section (same case as Fig. 1); APAAP; haematoxylin counterstain; ×520

Table 2. Correlation of the results of latent membrane protein expression with vimentin immunostaining on Hodgkin's and Reed-Sternberg cells and the findings from in situ hybridization for the detection of EBV genome

Histological subtype (Total)			EBV DNA		Vimentin	
			+	−	+	−
LP (5)	LMP−	5	0	5	0	5
NS (20)	LMP+	2	2	0	2	0
	LMP−	18	3	15	11	7
MC (11)	LMP+	6	6	0	6	0
	LMP−	5	1	4	2	3
LD (3)	LMP+	3	3	0	3	0

LMP, latent membrane protein; LP, lymphocyte predominance; NS, nodular sclerosis; MC, mixed cellularity; LD, lymphocyte depletion; ND, not done; +, positive, −, negative

the presence of EBV nucleic acids; therefore, 4 EBV nucleic acid positive cases were LMP negative (Table 2).

Vimentin expression on HRS cells was found in all the 11 LMP-positive cases (Fig. 4); visualization of HRS cells with the anti-LMP mAbs on adjacent sections revealed that the proportion of vimentin-positive cells was in the range between 30% and 90%. Only in 2 cases was the number of LMP-expressing HRS cells lower than that of vimentin-positive HRS cells (Table 1). Vimentin expression was detected in 13 additional cases

(11 of the NS subtype and 2 of the MC subtype that were negative for LMP expression. Thus, co-expression of LMP and vimentin was found in 11 cases, mostly of the MC and LD subtypes, whereas HRS cells expressed vimentin in 13 of 28 (46.4%) LMP-negative cases, mostly of the NS subtype. Interestingly, vimentin expression was detected in 4 cases that were negative for LMP expression but positive for EBV genomic material detection by ISH (see Table 1).

Discussion

Different techniques including Southern blot, PCR and ISH have demonstrated a significant association between EBV genome and HD, suggesting a possible role for the virus in the development of a proportion of cases. It is noteworthy that HRS cells in EBV-positive cases have been shown to express a latent infection protein phenotype (LMP^+, $EBNA2^-$) which differs from that of other EBV-associated lymphomas (Delsol et al. 1992; Herbst et al. 1991; Pallesen et al. 1991). Recently, it has been suggested that LMP expression detection may be adequate and technically simpler for identifying EBV-associated HD (Delsol 1992).

In this study, both ISH and LMP immunohistochemical staining were used for identifying HD cases in which EBV is present in the HRS cells, a prerequisite for assuming any role for the virus in the neoplastic process.

All 39 cases selected for study showed histological and immunohistological features consistent with the conventional diagnosis of HD. In 9 cases HRS cells expressed B- or/and T-cell-associated antigens, while in a significant proportion of cases HRS cells were found to be negative for the lymphoid-associated antigens, and ISH studies for detecting immunoglobulin light chain mRNA revealed no staining of HRS cells.

In this study, the demonstration of EBV in HRS cells in 38.4% of the cases is comparable with the findings of other reports (Boiocchi et al. 1992; Delsol et al. 1992). ISH revealed EBV in 3 of 3 (100%) cases of LD, 7 of 11 (64%) cases of MC and 5 of 20 (25%) cases of NS. These findings are consistent with the frequency of EBV positivity that has been identified by PCR on fixed tissues in HD (Shibata et al. 1991). In the study of Shibata et al. (1991) EBV was most often detected in the subtypes of MC (12 of 15 cases), NS (7 of 14 cases), and lymphocyte depletion (5 of 7 cases) compared with nodular LP HD (2 of 14 cases). In contrast with specimens with infectious mononucleosis and progressively transformed germinal centres, only one EBV genotype was evident in the specimens with HD, supporting the hypothesis that some cases of HD may be directly associated with EBV (Shibata et al. 1991).

LMP expression was found in 28.2% of the cases, only in the presence of EBV genome detection; however, LMP was expressed only by 11 of the 15 (73.3%) cases which showed EBV nucleic acids by ISH. Such discrepancies between the presence of EBV DNA detected by PCR or ISH and the absence of LMP expression have been reported (Delsol et al. 1992; Herbst et al. 1991).

Considering LMP expression alone, the percentage of positive cases in this study is lower than that of Pallesen et al. (1991) (48%; 40/84), Herbst et al. (1991) (38%; 18/47), and Murray et al. (1992) (48%; 22/46), but rather similar to that of Delsol et al. (1992) (33.5% versus 28.2% of this study). The lower positivity for LMP as compared with the other studies may be traced back to the use of Bouin-fixed tissue sections. Anti-LMP antibodies were tested on Bouin-fixed material in the study of Delsol et al. (1992) as well as in the present work, while cryostat sections were used in the studies of Pallesen et al. (1991) and Herbst et al. (1991); formol-saline-fixed paraffin-embedded tissues were used by Murray et al. (1992).

The present data show a higher association of the LMP expression with the "aggressive" LD (3 positive out of 3 cases; 100%) and MC (6 positive out of 11 cases; 54.5%) histological subtypes than with LP (0 positive out of 5 cases) or NS (2 positive out of 20 cases; 10%) subtypes. The distribution of LMP positivity within the subtypes is similar to that reported by Delsol et al. (1992). Moreover, the frequency of LMP-positive cases of the MC subtype is consistent with that recently found by Herbst et al. (1991) (50% versus 54.5% in this study). Conversely, we found a lower percentage of LMP-positive NS cases (10% versus 33% in the Herbst series). A higher association of LMP expression with the MC subtype (96%) than with NS (32%) and LP (10%) was found by Pallesen et al. (1991).

With regard to vimentin expression on HRS cells, the present data confirm previous studies reporting on the heterogeneous expression of vimentin in HD (Carbone et al. 1990b; Tamaru et al. 1990). In addition, in this study, the expression of vimentin on HRS cells was found in all the LMP-expressing cases and only in a fraction of LMP-negative cases. This finding is not surprising because it has been indicated that LMP is associated with the cytoskeletal protein vimentin in EBV-infected, transformed lymphocytes (Liebowitz et al. 1987). Moreover, it seems that LMP and vimentin often co-localize in a single patch near the plasma membrane (Liebowitz et al. 1987). In light of the transforming potential of LMP and the interaction between vimentin and LMP (Birkenbach et al. 1989), the association of LMP and vimentin expression on HRS cells, as emphasized in this study, provides further evidence for a significant role of EBV in the development of a proportion of HD cases. The meaning of vimentin expression on HRS cells in LMP-negative cases in uncertain. In some cases it may reflect a technically undetectable LMP expression: in 4 vimentin-positive and LMP-negative cases (nos. 7, 9, 10, 31; see Table 1) in which ISH demonstrated EBV genome in HRS cells, LMP negativity was probably due to the fact that HRS cells did not express LMP levels detectable by immunohistology. Such an explanation may also be considered for the 14 LMP-negative HD cases of the series studied by Herbst et al. (1991), in which HRS cells expressed vimentin and PCR analysis for EBV-specific DNA sequences was positive. In the other vimentin-expressing but LMP and EBV DNA negative cases (15/47 of the series of Herbst et al.;

9/39 of this series), however, other mechanisms need to be considered for vimentin expression on HRS cells (Ferrari et al. 1986).

We conclude that the results of this study further support the view that HD represents a heterogeneous group of disorders and extend this to EBV association; the percentage of positive cases ranging from 38.4% by ISH analysis to 28.2% by EBV LMP immunohistological detection. The immunohistological demonstration of LMP provides a simple approach for analysis of EBV genome expression in HD and the expression of LMP exclusively by EBV-containing HRS cells is evidence for functional activity. The expression of this protein is of particular interest since, besides promoting neoplastic transformation and inhibiting terminal differentiation, LMP can serve as a target for cytotoxic T-lymphocyte recognition. Further, LMP expression was strongly related to the "aggressive" LD and MC histological subtypes of HD, suggesting that they may be EBV-associated diseases. Nearly all LMP-positive cases (10/11) included HD specimens in which HRS cells were without expression of B- or T-cell markers. Moreover, light chain mRNA was not detected by ISH in HRS cells. Finally, LMP expression was strongly related to vimentin expression on HRS cells. The co-expression of LMP and vimentin on HRS cells may suggest that LMP is associated with this cytoskeletal protein and can induce increased vimentin levels also in HRS cells. Other mechanisms may be suggested for explaining vimentin expression on HRS cells in LMP and EBV DNA negative cases.

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