PERGAMON

European Journal of Cancer 37 (2001) 1276–1287

European Journal of Cancer

www.ejconline.com

Pathogenetic and histogenetic features of HIV-associated Hodgkin's disease

R. Dolcetti [a], M. Boiocchi [a,*], A. Gloghini [b], A. Carbone [b]

[a] *Division of Experimental Oncology 1, Centro di Riferimento Oncologico, IRCCS, National Cancer Institute, via Pedemontana Occidentale 12, 33081 Aviano (PN), Italy*

[b] *Division of Pathology, Centro di Riferimento Oncologico, IRCCS, National Cancer Institute, via Pedemontana Occidentale 12, 33081 Aviano (PN), Italy*

Received 5 February 2001; accepted 7 March 2001

Abstract

Compared with the cases in the general population, Hodgkin's disease (HD) arising in the HIV setting shows distinctive features in terms of epidemiology, aetiopathogenesis, histopathology and clinical behaviour. Although HD does not represent an AIDS-defining condition, recent evidence consistently indicates that HIV-infected individuals have a significantly increased risk of developing HD. HIV-related HD is characterised by the preponderance of aggressive histological subtypes, advanced stage at presentation, and highly malignant clinical course. Moreover, unlike HD in the general population, the large majority of HIV-related HD cases are pathogenetically linked to Epstein–Barr virus (EBV), with rates of EBV positivity ranging from 80 to 100%. Hodgkin and Reed-Sternberg cells of these cases invariably show a strong expression of the EBV-encoded latent membrane protein-1 (LMP-1), which functions as a constitutively activated tumour necrosis factor (TNF) receptor-like molecule. Usurpation of physiologically relevant pathways by LMP-1 may lead to the simultaneous or sequential activation of signalling pathways involved in the promotion of cell activation, growth, and survival, contributing thus to most of the features of HIV-related HD.

Keywords: Hodgkin's disease; HIV; Epstein–Barr virus; CD40; LMP-1

1. Epidemiology

The epidemiological features of HIV-unrelated Hodgkin's disease (HD) suggest that this malignancy is probably a heterogeneous disorder including different clinico-pathological entities. In fact, unlike most other tumours, HD of HIV-seronegative patients shows a bimodal age-incidence curve which is characterised, in Western countries, by a first peak between the ages of 15 and 34 years and a second peak in older adults [1,2]. Nodular sclerosis (NS) HD accounts for the young adult peak, whereas the incidence of the other HD histotypes progressively increases with age [1,2]. In contrast, in underdeveloped areas of the world, incidence rates are generally higher in childhood with a less pronounced young adult peak and a relatively higher prevalence of the mixed cellularity (MC) subtype [2,3]. In developing nations, all three age peaks of disease may be encountered, with a wide spectrum of clinicopathological features [1–3]. These differences in terms of geographical and socioeconomic factors showed by HIV-unrelated HD must be taken into account when analysing the epidemiological characteristics of HIV-associated HD.

At the outset of the AIDS epidemic, only isolated cases or small series of patients with HIV-associated HD have been reported, with incidence values markedly lower than those observed for non-Hodgkin's lymphomas (NHLs) that have arisen in the HIV setting. On these grounds, HD has not been included among the AIDS-defining illnesses. Besides the relative low incidence of the disease, the definition of the relationships between HD and HIV infection was also complicated by possible histopathological misclassification and the relatively high incidence of HD in the age group most at risk for HIV infection [1,2]. Subsequent analyses revealed a slight increase of HD among homosexual men from San Francisco, although no trend towards an increased

* Corresponding author. Tel.: +39-434-659300; fax. +39-434-659659.

E-mail address: mboiocchi@cro.it (M. Boiocchi).

0959-8049/01/$ - see front matter
PII: S0959-8049(01)00105-8

incidence of the disease could be demonstrated [4]. More recently, several cohort and registry-linkage investigations, mostly from the United States, Europe, and Australia, consistently reported a significant excess of HD (2.5- to 38-fold) among individuals with or at risk of AIDS [5–13] (Table 1), thus supporting the hypothesis that HIV-related immunosuppression increases the risk of the development of HD. However, a similar increase in the incidence of HIV-related HD was not observed in other areas of the world, particularly in Africa [14–16]. It remains to be elucidated whether, in these countries, a different interplay between the host and the factors underlying HD pathogenesis may account for these findings. It can not be ruled out, however, that the lack of increased HD incidence in HIV-infected African populations may be consequent to a masking effect due to other and more relevant HIV-associated causes of death in these regions of the world.

Early studies from Europe, particularly Italy, Spain and France, suggested that HIV-related HD occurred in a similar epidemiological setting [17–20]. In these countries, intravenous (i.v.) drug addicts represented the predominant high-risk group [17–20]. An increased incidence of HD was also reported among i.v. drug users in New York prisons [21]. Although the reasons for such a correlation are still a matter of debate, these findings are consistent with the possibility that transmission of blood-borne infectious agents may be involved in the pathogenesis of HIV-related HD in i.v. drug addicts. It can not be excluded that the possible immunomodulatory activity of still unidentified substances contaminating the injected drugs may also contribute to the development of HD in these patients. However, recent studies have indicated that the increased risk of HD is not restricted to i.v. drug users, but has also been found to be significantly higher in homosexual/bisexual men [5,7,9,13]. Moreover, an increased incidence of HD was also found in HIV-infected haemophiliacs, although the excess was not statistically significant [22]. Further studies including larger series of HD cases will be required to fully understand the complex relationship between HIV-associated immunosuppression and the development of HD.

2. Histological types and conventional phenotypes of Hodgkin's disease

Nodular lymphocyte predominance HD (NLP HD) and classic HD (CHD) are the two main categories of HIV-unrelated HD [23–25]. According to the recent World Health Organization (WHO) proposal [26], CHD includes NS, lymphocyte rich, MC, and lymphocyte depletion subtypes (Table 2). HD in HIV-infected individuals mainly includes the aggressive histological subtypes of CHD, namely MC, lymphocyte depletion,

Table 2
Proposed World Health Organization Classification of Hodgkin's disease [26]

→ **Nodular lymphocyte predominance**
→ **Classic Hodgkin's disease**
Nodular sclerosis
Lymphocyte rich
Mixed cellularity
Lymphocyte depletion

Table 1
Relative risk of HD in people with or at risk of AIDS from cohort and registry-linkage studies

Author [Ref.]	Country	Type of study	No. of HD cases	RR (95% CI)
Hessol and colleagues [5]	United States (San Francisco)	Cohort: 6704 homosexual men (1978–1989)	8	5.0 (2.0–10.3)
Reynolds and colleagues [6]	United States (San Francisco)	Linkage of data from AIDS and Cancer registries (1980–1987)	16	8.8 (5–14.3)
Lyter and colleagues [7]	United States (Pittsburgh)	Cohort: 1199 homosexual men (1984–1993)	2	19.8 (2.4–71.5)
Koblin and colleagues [8]	United States (San Francisco, New York)	Cohort: 15 565 homosexual men (1978–1990)	18	2.5 (1.5–3.9)
Serraino and colleagues [9]	Italy	Cohort: HIV seroconverters	3	38 (7.8–111)
Goedert and colleagues [10]	United States and Puerto Rico	Linkage of data from AIDS and Cancer registries	140	7.6 (4.1–13.1)
Franceschi and colleagues [11]	Italy	Linkage of data from AIDS and Cancer registries	11	8.9 (4.4–16.0)
Grulich and colleagues [12]	Australia	Linkage of data from AIDS and Cancer registries	9	18.3 (8.4–34.8)
Serraino and colleagues [13]	Italy and France	Cohort: DMI-2 HIV (Nice, 1988–1998), Italian HIV seroconverters, and San Patrignano community (Italy) databases	7	8.7 (3.4–18.0)

HD, Hodgkin's disease; RR, relative risk; CI, confidence interval.

and Reed-Sternberg (RS) cell-rich cases, whereas NLP HD is uncommon [27,28].

The neoplastic cells of NLP HD are termed lymphocyte and histiocyte (L&H) cells, whereas the neoplastic cells of CHD are defined as RS cells. L&H cells of NLP HD and RS cells of CHD have different morphology, different phenotype and different infection pattern by Epstein–Barr virus (EBV) [25,29,30]. L&H cells express CD20, CD45 and EMA antigens, whereas RS cells display a CD15-positive, CD30-positive and CD45-negative phenotype [25,29–32]. EBV infection is usually present only in RS cells, which express the EBV-encoded latent membrane protein-1 (LMP-1) [25,33]. In the AIDS-setting, RS cells are characterised by a high frequency of EBV association (from 80 to 100%) [27,28,34].

In the general population, NLP HD and CHD differ not only in the nature of the neoplastic population, but also with respect to the surrounding reactive T cells, which display distinct phenotypes. In fact, T cells in NLP HD express CD57 [29,30,35], but not CD40L [36,37]. Conversely, T cells in CHD display a CD40L-positive phenotype [36]. It is important to remember that CHD occurring in HIV-infected people exhibits pathological features of the cellular background that differ from those of HD seen in the general population. In fact, the pathological spectrum of HIV-related HD typically includes the presence of fibrohistiocytoid stromal cell proliferation and inversion of the CD4+/CD8+ T-cell ratio [28,33,34].

3. CD40 expression on RS cells

The expression of CD40L by reactive T cells in HIV-unrelated CHD is crucial because CD40L is an activation antigen of T cells acting as a specific ligand for CD40 [38]. CD40, which belongs to the Tumour Necrosis Factor (TNF)/Nerve Growth Factor (NGF) receptor superfamily and is involved in B-cell growth and differentiation [38], is consistently expressed on RS cells [39–41] and is functionally active both as an adhesion molecule and as a growth signal transducer [39]. Since CD40L-positive T lymphocytes are mainly located close to RS cells [36], it has been suggested that the interaction between RS cells and the microenvironmental T cells may be mediated in part by the CD40/CD40L adhesion pathway [36].

With regard to the reactive cellular background, the overwhelming majority of HIV-related HD cases demonstrate inversion of the $CD4^+/CD8^+$ T-cell ratio in all morphological subtypes and in all conventional phenotypes of the disease [42]. Among reactive T cells in the background, expression of CD40L occurs only rarely. In particular, the rare $CD40L^+$ T lymphocytes are distributed in a scattered fashion in the tumour tissue and no rosetting of RS cells by $CD40L^+$ T lymphocytes can be detected. Overall, these data suggest that stable CD40/CD40L interactions between RS cells and reactive T lymphocytes is not a feature of HIV-related HD [42].

4. Clinical features

In addition to EBV association and histopathological features, the clinical correlates of HD in HIV-infected patients are also different from those of HD in the general population. In fact, the majority of HIV-seropositive patients with HD show a widespread disease at presentation [18,20,27,43], similarly to what is observed in patients with AIDS-related Non-Hodgkin's lymphomas (NHLs). Unlike HIV-unrelated HD, HIV-seropositive patients may show a non-contiguous spread of the disease with a frequent involvement of extranodal sites [18,20,27,43]. Of note, approximately 40–50% of the cases show HD infiltrating the bone marrow, with this site being the first presentation of HIV-related HD in approximately 20% of the cases [18,20,27,43]. Moreover, systemic 'B' symptoms, including fever, night sweats, and/or weight loss, occur more frequently in patients with HIV-related HD, with prevalence values ranging from 70 to 100% [17,18,20,43,44]. For a recent, more comprehensive review of the clinical characteristics of HIV-related HD see the paper by Vaccher and colleagues in this issue [45].

5. The pathogenetic association with EBV

Evidence accumulated so far indicates that, in Western countries, EBV is causally involved in the development of approximately 40% of HD cases in HIV-seronegative patients (reviewed in [46,47]). In developing areas of the world, HIV-unrelated HD usually show higher rates of EBV positivity, suggesting a role for geographical and/or ethnic factors in determining the incidence and association of EBV with the disease [48–50]. Moreover, HIV-unrelated HD shows a strong correlation between histological subtype and EBV, with the MC histotype being more likely EBV-associated than NS cases [3,46,47]. With regard to age, older adult and childhood HD of HIV-seronegative patients were found to be more frequently associated with EBV than the young adult cases [3,46,47].

HD cases from HIV-infected patients are markedly different from those in the general population in terms of association of the EBV. In fact, data from all series investigated so far consistently showed that the large majority of HIV-related HD cases are pathogenetically linked to EBV, with rates of EBV positivity ranging from 80 to 100% of the cases [27,50–53]. Early studies

showed that virtually all Hodgkin's and RS cells from HIV-associated HD cases harboured EBV DNA sequences, thus providing direct evidence of a causal relationship with the virus [51,54]. These results were later confirmed by a more sensitive *in situ* approach based on the detection of the EBV-encoded small nuclear RNAs, the EBERs, viral transcripts that are generally present in high copy numbers in latently infected cells [27,50,52,53,55]. Similar to what was observed for HIV-unrelated HD, analysis of the configuration of the terminal repeat region of the EBV genome by Southern blot hybridisation showed the presence of clonal EBV episomes in HD lesions from HIV-seropositive patients [56,57]. These findings are consistent with the presence of a monoclonal expansion of EBV-carrying cells in most HIV-associated HD and indicate that EBV infection takes place before the clonal expansion of the Hodgkin's and RS cells. It is highly unlikely that the presence of EBV genomes in HD-involved tissues reflects the expansion of EBV-infected lymphoblastoid cell clones favoured by the reduced immunocompetence associated with HIV infection. In fact, a single EBV episome was detected in the large majority of HIV-related HD cases investigated so far [27,56]. Moreover, we have demonstrated that a single EBV-infected cellular clone, with morphological features of Hodgkin's and RS cells, persists in multiple metachronous localisations of the disease [57]. These results argue strongly in favour of a functional role of the EBV-carrying clone and lend further support to the hypothesis of a causal involvement of the virus in the pathogenesis of most HIV-related HD cases.

6. Pattern of EBV-encoded gene expression

Analysis of EBV-encoded gene expression allows the distinction between latent or replicative EBV infection. In immunocompetent patients with EBV-related HD, the virus harboured by the Hodgkin's and RS cells seems to be strictly latent, as shown by the expression of the EBERs and the absence of lytic antigens, such as gp350/220, VCA and EA [58]. Moreover, expression of the BZLF1 trans-activator was found in the nuclei of a very small proportion of Hodgkin's and RS cells and only in few HIV-unrelated HD cases, indicating that when activation of the replicative cycle occurs, this usually results in abortion of virus production [59]. Although available evidence indicates that profound immunodeficiency may allow EBV to exit from latency and actively replicate [60], it has been convincingly demonstrated that EBV is also strictly latent in Hodgkin's and RS cells from HIV-related HD [61]. These results differ from findings in other EBV-related NHLs in immunodeficient patients, in which up to 60% of cases showed evidence of EBV replication [62]. These discrepancies may be due to differences in the immune status of the host, type and/or differentiation status of EBV-infected cells, microenvironmental signalling, or virus-specific properties.

In EBV-related HD from HIV-seronegative patients, Hodgkin's and RS cells almost invariably express EBERs, Epstein–Barr Virus Nuclear Antigen (EBNA)-1, LMP-1 and LMP-2, but not EBNA-2, a pattern of viral latency superimposable to that detected in nasopharyngeal carcinoma (NPC) and in some T-NHLs (reviewed in [63,64]). These similarities are probably dependent on the use of the same virus promoters by the EBV-carrying cells sustaining these diseases. This form of EBV latency, called latency II, is clearly distinct from that observed in other EBV-related disorders. In fact, a more restricted pattern of EBV latency characterises Burkitt's lymphoma cells, in which only the EBERs and EBNA-1 are expressed (latency I) [63,64]. However, EBV-immortalised lymphoblastoid B-cell lines and B-NHLs of immune compromised patients usually express the full set of EBV-encoded latent genes (latency III) [63,64]. As for EBV-related HD in the general population, Hodgkin's and RS cells of HIV-associated HD also express LMP-1 but not EBNA-2, indicating that the virus also adopts a latency II pattern in these cases [27,34,52,56,65]. Interestingly, in spite of the HIV-related immunosuppression, Hodgkin's and RS cells of these cases showed no partial or total shift towards a broader form of EBV latency, as observed in some B-NHL from HIV-seropositive patients [66]. This may be related to different degrees of immunosuppression underlying these conditions. In fact, HD is an earlier manifestation of HIV infection than NHLs and these patients usually show higher $CD4^+$ cell counts and a significantly lower prevalence of an AIDS-defining disease prior to the onset of HD [17,18,20,27].

7. Functional and transforming properties of LMP-1

Although recent findings have indicated that EBERs [67] and EBNA-1 [68] may contribute to EBV-induced transformation, it seems unlikely that these EBV-encoded gene products play a major role in the pathogenesis of HIV-related HD. In fact, both EBERs and EBNA-1 are expressed in virtually all cells latently infected with EBV, including normal EBV-carrying lymphocytes of long-term carriers [63,64]. In contrast, LMP-1 is not stably expressed in physiological conditions and is characterised by functional properties that may be relevant to the pathogenesis of the disease [63,64]. Early studies have identified *LMP-1* as a viral oncogene as its protein product is able to transform rodent cells. In fact, fibroblasts constitutively expressing LMP-1 have reduced serum requirements, grow in soft agar, lose contact inhibition, and become tumorigenic in nude

mice [69–71]. Moreover, EBV recombinant genetic analyses have demonstrated that LMP-1 is critical for primary B lymphocyte immortalisation [72]. In these cells, LMP-1 can also induce many of the phenotypic changes associated with EBV infection, including increased homotypic adhesion and upregulation of adhesion molecules leucocyte function antigen (LFA)-1, Intercellular Adhesion Molecule (ICAM)-1, LFA-3) [73,74], B-cell activation markers (CD23, CD30, CD40, CD71) [75,76] and anti-apoptotic genes (*Bcl-2*, *BclxL*, *Mcl1*, *A20*) [77–79]. In addition to contributing to the induction of some phenotypic features of H-RS cells, LMP-1 expression may be partly responsible for the peculiar cytomorphological features of these cells. In fact, it has been shown that LMP-1 interacts with cytoskeletal proteins such as vimentin [80,81] and that LMP-1-mediated CD99 downregulation induces the generation of cells with the H-RS cell phenotype [82].

Structurally, LMP-1 is a phosphoprotein of 386 amino acids characterised by a short amino-terminal sequence, six membrane-spanning domains, and a long carboxy-terminal cytoplasmic tail. The hydrophobic transmembrane domains are responsible for the oligomerisation of LMP-1 molecules in the plasma membrane, and mutations in this portion of the protein severely impair LMP-1-mediated transformation [83]. This is probably due to the fact that aggregation enables the cytoplasmic domains of several LMP-1 molecules to locally concentrate next to the plasma membrane, thus mimicking the oligomerisation of growth factor receptors occurring after interaction with the specific ligand. Indeed, molecular analyses demonstrated that aggregation of LMP-1 molecules in the plasma membrane causes at least three specific sites within the LMP-1 carboxy-terminal domain to constitutively mediate essential transforming signals. Two of these signalling domains, termed C-terminal activating region 1 and 2 (CTAR1 and CTAR2), were shown to interact with TNF receptor-associated factors (TRAFs) [84,85] and TNF receptor-associated death domain protein (TRADD) [86,87], respectively (Fig. 1). Association of these factors with LMP-1 accounts for the induction of the transcription factors NF-κB and AP-1 [84–87], which are key regulators of genes involved in cell activation and growth control [88,89]. A third activating region, located between CTAR1 and CTAR2, was recently shown to mediate recruitment and activation of Janus Kinase 3 (JAK3) ultimately resulting in the triggering of the JAK/STAT pathway [90] (Fig. 1). This signalling pathway is of crucial importance in several cellular decisions, such as proliferation and protection from apoptosis [91]. These findings clearly indicate that LMP-1 shares functional properties with members of the TNF receptor superfamily, and particularly with CD40 [74,92]. Nevertheless, unlike TNF receptor molecules, LMP-1 engages at least part of the CD40 pathway in a ligand-independent manner. Induction of NF-κB and AP-1 and activation of the JAK/STAT pathway probably account for many of the cellular changes observed in response to LMP-1. More importantly, usurpation of these physiologically relevant pathways by LMP-1 likely contributes to the pathogenesis of most EBV-associated disorders through the simultaneous or sequential activation of signalling pathways involved in the promotion of cell activation, growth, and survival.

The pathogenetic relevance of LMP-1 is also highlighted by other functional properties ascribed to this protein, suggesting that LMP-1 may favour the escape of H-RS cells from the host immune responses. In fact, transfection of the *LMP-1* gene into neoplastic B-cell lines results in upregulated production of interleukin-10 (IL-10) [93], a cytokine known to inhibit T-cell responses. Consistently high levels of IL-10 expression were found in HIV-unrelated EBV-positive HD cases [94]. Moreover, intact LMP-1, as well as peptides derived from the first transmembrane region of the protein, directly inhibit T and Natural Killer (NK) cell function [95]. These findings, together with the observation that LMP-1 may be actively secreted by EBV-infected cells, point to a role of LMP-1 in the local inhibition of EBV-specific T-cell responses observed in EBV-associated cases in the general population [96]. Little is known about the nature and extent of immune dysfunction in patients with HIV-related HD. Further studies are therefore required, particularly to assess whether LMP-1 also has a role in affecting local immune responses in HD cases from HIV-seropositive individuals.

8. EBV subtypes and variants in HIV-related HD

Recent molecular studies have highlighted the existence of a significant degree of sequence variations among naturally occurring EBV strains. In particular, differences within the genomic regions encoding for EBNA-2,-3,-4 and 6 allow the distinction between two different EBV subtypes, called type A and B (or type 1 and 2) (reviewed in [97]). These sequence differences are probably responsible for the different biological properties of the two EBV subtypes, as suggested by the observation that type A EBV has more efficient *in vitro* transforming activity than type B virus [98]. Type A is widespread in healthy Western populations, whereas type B has been associated with populations in Equatorial Africa [98]. Besides geography, however, other factors affect the epidemiology of these subtypes. In fact, type B EBV has been frequently detected in throat washings from healthy individuals and in peripheral blood of immunocompromised patients from Western countries, including HIV-seropositive individuals [98]. The studies aimed at characterising the EBV strain present within HD-involved tissues showed that type A

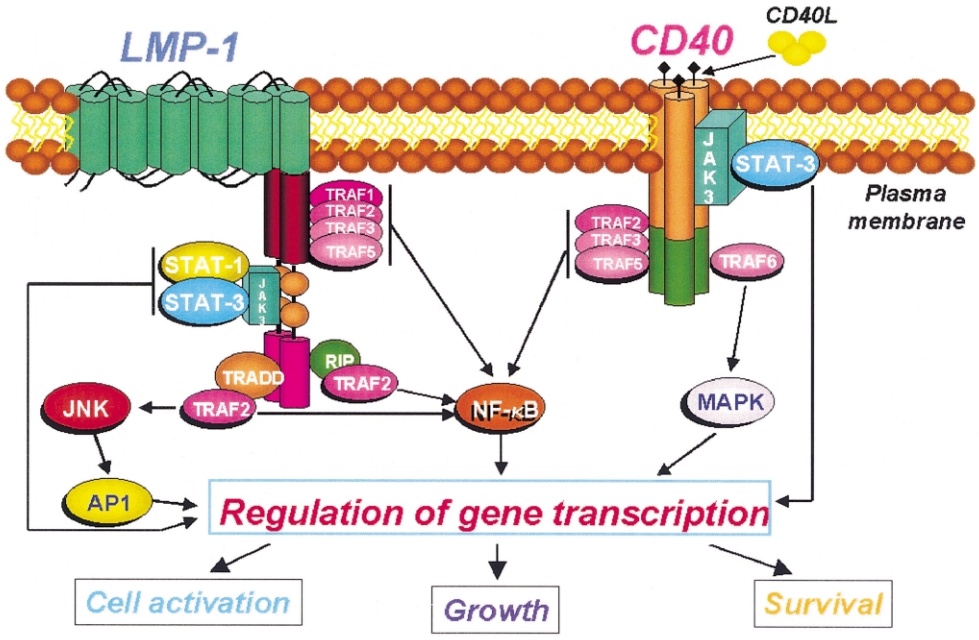

Fig. 1. Schematic representation of the known signalling pathways activated by the EBV-encoded LMP-1 in comparison with those activated by CD40 triggering.

EBV was largely predominant in HIV-unrelated cases, with prevalence values ranging from 56 to 100% [99–102]. In contrast, approximately 50% of HD cases from HIV-seropositive patients carried type 2 EBV [100,101]. These findings are consistent with those obtained in EBV-associated NHLs of HIV-seropositive patients [103] and collectively indicate that, in the context of HIV-mediated immunosuppression, both EBV subtypes are equally capable of contributing to the development of HD.

EBV sequence variations have been also detected within the 3′ end of the *LMP-1* gene, encoding for the carboxy terminal domain of the protein [97]. In particular, a 30-bp deletion in this portion of the *LMP-1* gene has been detected in 10–40% of HD from HIV-seronegative patients [104–107]. This deletion results in the absence of 10 amino acids and is identical to that observed in most Asian nasopharyngeal carcinomas [108,109]. Transfectants expressing the 30-bp *LMP-1* deletion were shown to have increased tumorigenicity and reduced immunogenicity compared with cells carrying a full-length gene [110,111]. Moreover, these deletion mutants are able to stimulate NF-κB-mediated transcriptional activation [112] and may enhance the half-life of the protein [113]. These findings, coupled with the observation that HD cases with *LMP-1* deletions have clinico-pathological features of more aggressive diseases [104–106], suggested that 3′ *LMP-1* deletions could enhance the transforming properties of EBV and increase the malignancy of EBV-induced disorders. More recently, however, we and others have demonstrated that *LMP-1* deletion mutants are relatively frequent in European healthy carriers, with a prevalence comparable to that of EBV strains with full-length *LMP-1* [107,114]. These findings suggest that infection with EBV variants carrying *LMP-1* deletions does not confer an increased risk of developing HD in the general population. With regard to the HIV setting, *LMP-1* deletions were found as frequently as the full-length gene in normal peripheral blood mononucleated cells of HIV-seropositive individuals, indicating that HIV-related immunosuppression does not seem to increase the prevalence of *LMP-1* deletion mutants [107]. A different picture is seen from the analysis of HIV-related HD cases, which repeatedly showed a significantly higher prevalence of *LMP-1* deletions when compared with HIV-unrelated HD [106,107]. Considering the peculiar clinico-pathological features of HIV-related HD, the possibility that *LMP-1* deletions may contribute to the malignant behaviour of these disorders constitutes an attractive hypothesis that deserves further investigation.

9. Histogenesis of HIV-unrelated and HIV-related Hodgkin's disease

In parallel to the molecular investigations, further insights into HD histogenesis have been gained by the use of biological markers identifying distinct subsets of mature B-cells. Two such markers are the BCL-6 protein and the CD138/syndecan-1 antigen, which may discriminate between germinal centre (GC) and post-GC B cells [115]. It has been showed that within the

mature B-cell compartment, expression of BCL-6, a zinc finger transcriptional repressor, clusters with GC B cells. Conversely, syndecan-1 (syn-1), a proteoglycan belonging to the syndecan family, is not expressed in GC B cells, but is expressed in post-GC B cells, including immunoblasts and plasma cells [115,116].

On these grounds, the combined expression pattern of BCL-6 and syndecan-1 was analysed in a large series of HD cases from the general population [117] and immunodeficient hosts with HIV infection [42]. These studies showed that tumour cells of NLP HD consistently express BCL-6, whereas they score negative for syn-1 and EBV infection in all cases. Concerning CHD, RS cells of the majority of HIV-unrelated CHD displayed the BCL-6$^-$/syn-1$^+$ phenotype of post-GC B cells, whereas the remaining fraction of HIV-unrelated CHD was constituted by a mixture of tumour cells reflecting the GC (BCL-6$^+$/syn-1$^-$) or post-GC (BCL-6$^-$/syn-1$^+$) phenotypes [117]. In contrast, most HIV-related HD cases expressed LMP-1 and displayed the BCL-6$^-$/syn-1$^+$ phenotype, thus reflecting post-GC B cells [42]. With regard to the reactive cellular background, BCL-6$^-$/syn-1$^+$ tumour cells of HIV-unrelated CHD were surrounded by T cells expressing CD40L [117]. Conversely, although BCL-6$^-$/syn-1$^+$ RS cells of HIV-related HD expressed CD40, they were not surrounded by CD40L$^+$ T lymphocytes [42].

Overall, these studies demonstrate that (1) L&H cells of NLP HD associate with the BCL-6$^+$/syn-1-phenotype [37,117]; (2) RS cell expression of the BCL-6$^+$/syn-1-phenotype is restricted to a minority of HIV-unrelated CHD [117] and is consistently absent in HIV-related CHD [42]; (3) the dominant phenotype in both HIV-unrelated and HIV-related CHD is represented by the BCL-6$^-$/syn-1$^+$ profile [42,117]; and (4) the phenotypical and biologic features of the reactive cellular background differ markedly in HIV-unrelated HD compared with HIV-related HD [42,117].

Interestingly, preliminary studies may provide a biological explanation for the phenotypic heterogeneity of RS cells of CHD. In fact, *in vitro* evidence shows that CD40/CD40L interactions and LMP1 expression can downregulate BCL-6 expression in B cells with the GC phenotype [118]. Thus, both events may represent a physiological negative regulatory signal for BCL-6, consistent with the hypothesis that downregulation of BCL-6 may be necessary for B cells to mature and exit the GC [118].

On these grounds, Fig. 2 summarises the proposed histogenetic model for the development of CHD in the general population. This model suggests that CD40/CD40L interactions modulate the differentiation of the neoplastic clone and, in accordance with *in vitro*

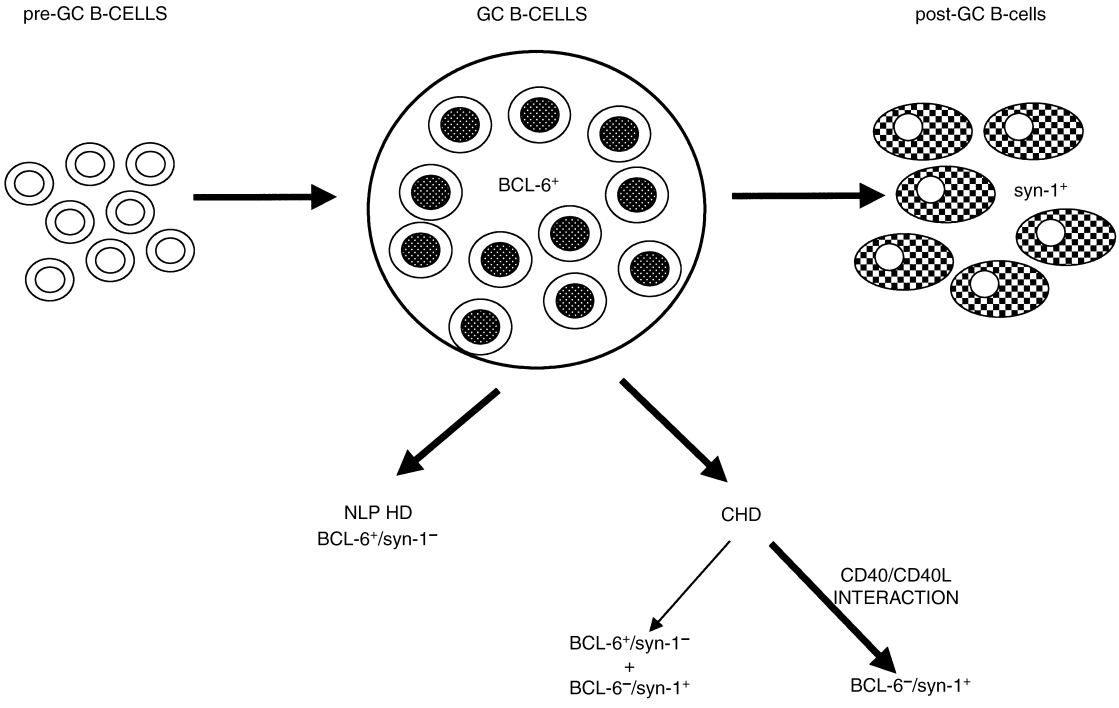

Fig. 2. Histogenetic model for the development of HD in the general population. This model is based on the combined expression pattern of BCL-6 and syn-1 throughout the stages of physiological B-cell differentiation. L&H cells of nodular lymphocyte predominance (NLP) HD consistently express the BCL-6$^+$/syn-1$^-$ phenotype and thus closely reflect the GC phenotype. Several CHD cases display only BCL-6$^-$/syn-1$^+$ RS cells, whereas a fraction of CHD displays some heterogeneity in the differentiation stage of the neoplastic clone. In these cases, CD40L$^+$ T cells preferentially cluster around BCL-6$^-$/syn-1$^+$ RS cells. This model suggests that CD40/CD40L interactions modulate the differentiation of the neoplastic clone and, in accordance with *in vitro* observations [118], downregulate BCL-6 in RS cells. CHD cases containing a mixture of BCL6$^-$/syn1$^+$ and BCL6$^+$/syn1$^-$ RS cells may represent tumours in which the differentiation process of RS cells is not complete in a fraction of cells.

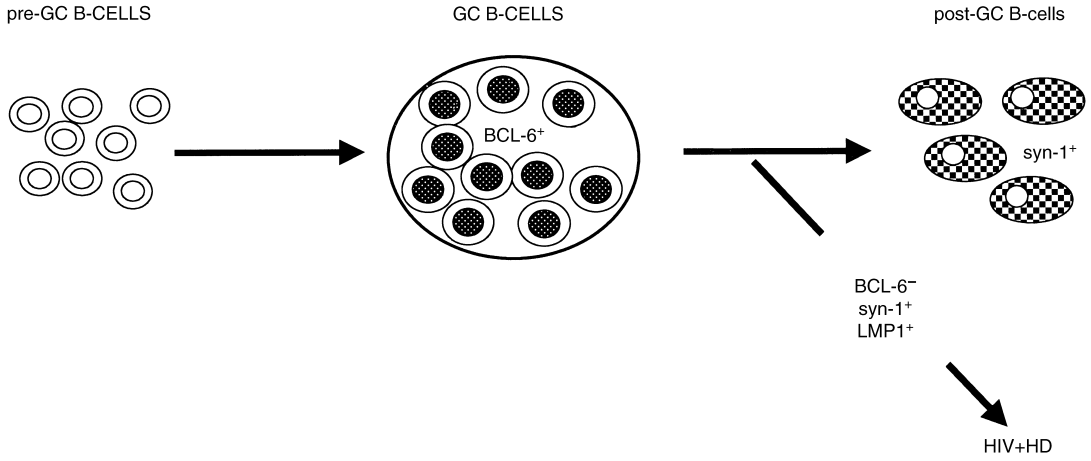

Fig. 3. Histogenetic model for the development of HD in individuals with HIV infection. In the HIV-setting, RS cells consistently express the BCL-6^-/syn-1^+ phenotype and thus reflect B cells which have transited through the GC and have undergone pre-terminal differentiation. It is assumed that the BCL-6^-/syn-1^+ phenotype is permissive for expression of LMP-1. This model suggests that LMP-1, which is functionally homologous to CD40, may contribute to the modulation of the RS cell phenotype in HIV-related HD. This is in contrast with HIV-unrelated HD, in which the phenotype of tumour cells is modulated by the surrounding cellular background, particularly by $CD40L^+$ reactive T cells.

observations, downregulate BCL-6 in RS cells, which therefore assume the BCL-6^-/syn-1^+ phenotype typical of post-GC B cells. Fig. 3 shows the proposed histogenetic model for the development of HD in individuals with HIV infection. RS cells reflect the post-GC phenotype also in this setting. However, it has been postulated that the acquisition of the BCL-6^-/syn-1^+ profile is not due to CD40/CD40L interactions, which are lacking in HIV-related HD, but rather is induced by the expression of the EBV-encoded LMP-1 antigen. In conclusion, these data indicate that the phenotype of tumour cells of HIV-unrelated CHD appears to be modulated by the surrounding cellular background, particularly by $CD40L^+$ reactive T cells. Conversely, RS cells of virtually all cases of HIV-related HD express LMP1, which, being functionally homologous to CD40, may contribute to the modulation of the HIV-related HD phenotype.

Novel histogenetic markers specifically associated with the late stages of B cell differentiation will conceivably provide a powerful tool to refine and corroborate the proposed model of HD histogenesis. Recently, MUM1/IRF4 (for multiple myeloma-1/interferon regulatory factor-4) [119] has been added to the panel of phenotypic markers available for the characterisation of B-cell lymphoma histogenesis since expression of MUM1/IRF4 protein appears to be strictly regulated during lymphoid differentiation and it is retained upon neoplastic transformation of B cells [119].

In reactive lymphoid tissues, the phenotypic patterns identified by MUM1, BCL-6, and syn-1 map to lymph node areas that are populated by B cells at different stages of differentiation. Comparison of the topography of MUM1 and BCL-6 within the GC reveals that expression of BCL-6 occurs immediately after a B cell enters the GC and is maintained until GC exit, whereas MUM1 positivity begins only at the centrocyte stage

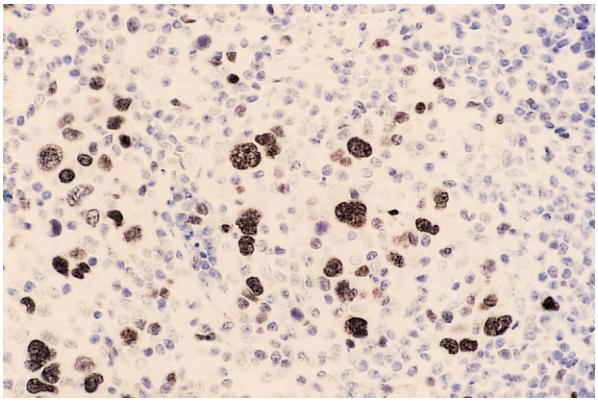

Fig. 4. In a lymph node involved by lymphocyte depletion HIV-related HD, MUM1 is expressed by a large number of tumour cells corresponding to RS cells and their variants. The staining is nuclear and intense. Immunoperoxidase, haematoxylin counterstain. Original magnification ×250.

and is maintained during post-GC maturation [120]. In this respect, most B cells within the GC, including all centroblasts and a large majority of centrocytes, express the BCL-6^+/MUM1^-/syn-1^- phenotype. A small fraction of GC B cells, located in the light zone of the GC and morphologically identifiable as a subset of centrocytes, express the BCL-6^-/MUM1^+/syn-1^- phenotype. Upon GC exit, B cells retain MUM1 expression and start to express syn-1, as documented by the observation that post-GC B cells undergoing maturation toward the plasma cell stage predominantly display the BCL-6^-/MUM1^+/syn-1^+ phenotype. On these grounds, the observation that RS cells of HIV-related HD consistently display the BCL-6^-/MUM1^+/syn-1^+ phenotype [120] corroborate the proposed concept that they reflect post-GC B cells (Fig. 4).

10. HD arising in patients with HIV-unrelated immunodeficiencies

While only little information is available on congenital immunodeficiencies, cases of HD have been reported in recipients of solid organ transplants [121–123], in patients undergoing allogeneic bone marrow transplantation (BMT) [124], and in those receiving immunosuppressive therapy for mixed connective tissue disease [125,126]. Similar to that which occurs in the HIV setting, HD is less frequently observed than NHL in these conditions of immune deficiency. In particular, BMT patients show a 6-fold increase in the risk for HD [124], whereas controversial data have been reported after solid organ transplantation [121,123]. Of note, the HD cases in patients with HIV-unrelated immunodeficiencies showed clinico-pathological and virological features similar to those of HIV-infected patients, with mixed cellularity subtype being predominant and most HD cases testing positive for EBV ($EBER^+$ and $LMP\text{-}1^+$) [121–126]. Moreover, in these patients, HD is usually diagnosed at advanced stages and tends to have an aggressive clinical course with an usually poor response to therapy [121–126]. It is also worth mentioning that in these cases, besides true HD cases, lymphoproliferations resembling HD may also occur [126,127]. An accurate histopathological evaluation of these cases is warranted, since a subset of these disorders will completely regress after discontinuation of the immunosuppressive regimen, thereby obviating the need for chemotherapy or radiation therapy. These findings support the notion that a still poorly defined impairment of immune responses contributes to the development of HD and should stimulate further studies aimed at elucidating the nature of these alterations in different clinical settings.

Acknowledgements

This work was supported by grants to R.D. and A.C. from the Italian Association for Cancer Research (AIRC). The authors thank Dr P. Tonel and Mrs P. Pistello for help with the manuscript.

References

1. MacMahon B. Epidemiology of Hodgkin's disease. *Cancer Res* 1966, **26**, 1189–1200.
2. Correa P, O'Conor GT. Epidemiologic patterns of Hodgkin's disease. *Int J Cancer* 1971, **8**, 192–201.
3. Macfarlane GJ, Evstifeeva T, Boyle P, Grufferman S. International patterns in the occurrence of Hodgkin's disease in children and young adult males. *Int J Cancer* 1995, **61**, 165–169.
4. Biggar RF, Burnett W, Mikl J, Nasca P. Cancer among New York men at risk of acquired immunodeficiency syndrome. *Int J Cancer* 1989, **43**, 979–985.
5. Hessol NA, Katz MH, Liu JY, Buchbinder SP, Rubino CJ, Holmberg SD. Increased incidence of Hodgkin's disease in homosexual men with HIV infection. *Ann Intern Med* 1992, **117**, 309–311.
6. Reynolds P, Saunders LD, Layefsky ME, Lemp GF. The spectrum of acquired immunodeficiency syndrome (AIDS)-associated malignancies in San Francisco, 1980–1987. *Am J Epidemiol* 1993, **137**, 19–30.
7. Lyter DW, Bryant J, Thackeray R, Rinaldo CR, Kingsley LA. Incidence of human immunodeficiency virus-related and non-related malignancies in a large cohort of homosexual men. *J Clin Oncol* 1995, **13**, 2540–2546.
8. Koblin BA, Hessol NA, Zauber AG, *et al*. Increased incidence of cancer among homosexual men, New York City and San Francisco, 1978–1990. *Am J Epidemiol* 1996, **144**, 916–923.
9. Serraino D, Pezzotti P, Dorrucci M, Alliegro MB, Sinicco A, Rezza G, *et al*. for the HIV Italian Seroconversion Study Group. *Cancer Incidence in a cohort of human immunodeficiency virus seroconverters Cancer* 1997, **79**, 1004–1008.
10. Goedert JJ, Coté TR, Virgo P, *et al*, for The AIDS-Cancer Match Study Group. Spectrum of AIDS-associated malignant disorders. *Lancet* 1998, **351**, 1833–1839.
11. Franceschi S, Dal Maso L, La Vecchia C. Advances in the epidemiology of HIV-associated non-Hodgkin's lymphomas and other lymphoid neoplasms. *Int J Cancer* 1999, **83**, 481–485.
12. Grulich AE, Wan X, Law MG, Coates M, Kaldor JM. Risk of cancer in people with AIDS. *AIDS* 1999, **13**, 839–843.
13. Serraino D, Boschini A, Carrieri P, *et al*. Cancer risk among men with, or at risk of, HIV infection in southern Europe. *AIDS* 2000, **14**, 553–559.
14. Lazzi S, Ferrari F, Nyongo A, *et al*. HIV-associated malignant lymphomas in Kenya (Equatorial Africa). *Hum Pathol* 1998, **29**, 1285–1289.
15. Parkin DM, Wabinga H, Nambooze S, Wabwire-Mangen F. AIDS-related cancers in Africa: maturation of the epidemic in Uganda. *AIDS* 1999, **13**, 2563–2570.
16. Chokunonga E, Levy LM, Bassett MT, *et al*. AIDS and cancer in Africa: the evolving epidemic in Zimbabwe. *AIDS* 1999, **13**, 1583–2588.
17. Monfardini S, Tirelli U, Vaccher E, *et al*. Malignant lymphomas in patients with or at risk for AIDS: a report of 50 cases observed in Italy. *Cancer Detect Prev* 1988, **12**, 237–241.
18. Andrieu JM, Roithmann S, Tourani JM, *et al*, for the French registry of HIV-associated Tumors. Hodgkin's disease during HIV1 infection: the French registry experience. French Registry of HIV-associated Tumors. *Ann Oncol* 1993, **4**, 635–641.
19. Roithmann S, Tourani JM, Andrieu JM. Hodgkin's disease in HIV-infected intravenous drug abusers. *N Engl J Med* 1990, **323**, 275–276.
20. Rubio R, for the Cooperative Study Group of Malignancies associated with HIV infection of Madrid. Hodgkin's disease associated with human immunodeficiency virus infection. A clinical study of 46 cases. *Cancer* 1994, **73**, 2400–2407.
21. Ahmed T, Wormser GP, Stahl RE, *et al*. Malignant lymphomas in a population at risk for acquired immune deficiency syndrome. *Cancer* 1987, **60**, 719–723.
22. Rabkin CS, Hilgartner MW, Hedberg KW, *et al*. Incidence of lymphomas and other cancers in HIV-infected and HIV-uninfected patients with hemophilia. *JAMA* 1992, **267**, 1090–1094.
23. Lukes R, Butler J, Hicks E. Natural history of Hodgkin's disease as related to its pathological picture. *Cancer* 1966, **19**, 317–344.
24. Warnke R, Weiss LM, Chan JKC, Cleary ML, Dorfman RF. Tumors of the lymph nodes and spleen. In *Atlas of Tumor Pathology*, third series, Fascicle 14. Armed Forces Institute of Pathology, 1995.
25. Harris NL, Jaffe ES, Stein H, *et al*. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. *Blood* 1994, **84**, 1361–1392.

26. Jaffe E, Harris NL, Diebold J, Diebold J, Muller-Hermelink H-K. World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues. A progress report. *Am J Clin Pathol* 1999, **111**(Suppl. 1), S8–S12.
27. Tirelli U, Errante D, Dolcetti R, *et al*. Hodgkin's disease and human immmunodeficiency virus infection: clinicopathologic and virologic features of 114 patients from the Italian Cooperative Group on AIDS and Tumors. *J Clin Oncol* 1995, **13**, 1758–1767.
28. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Human Immunodeficiency Viruses and Human T-Cell Lymphotropic Viruses, vol. 67. International Agency for Research on Cancer/World Health Organization, Lyon, 1996.
29. Harris NL. Hodgkin's disease: classification and differential diagnosis. *Mod Pathol* 1999, **12**, 159–176.
30. Mason D, Banks P, Chan J, *et al*. Nodular lymphocyte-predominance Hodgkin's disease: a distinct clinicopathological entity. *Am J Surg Pathol* 1994, **18**, 528–530.
31. Carbone A, Gloghini A, Volpe R. Paraffin section immunohistochemistry in the diagnosis of Hodgkin's disease and anaplastic large cell (CD30+) lymphomas. *Virchows Archiv (A)* 1992, **420**, 527–532.
32. Frizzera G, Wu CD, Inghirami G. The usefulness of immunophenotypic and genotypic studies in the diagnosis and classification of hematopoietic and lymphoid neoplasms. An update. *Am J Clin Pathol* 1999, **11**(Suppl. 1), S13–S39.
33. Carbone A, Weiss LM, Gloghini A, Ferlito A. Hodgkin's disease. Old and recent clinical concepts. *Ann Otol Rhinol Laryngol* 1996, **105**, 751–758.
34. Carbone A, Dolcetti R, Gloghini A, *et al*. Immunophenotypic and molecular analyses of acquired immune deficiency syndrome-related and Epstein–Barr virus-associated lymphomas: a comparative study. *Hum Pathol* 1996, **27**, 133–146.
35. Poppema S. The nature of the lymphocytes surrounding Reed-Sternberg cells in nodular lymphocyte predominance and in other types of Hodgkin's disease. *Am J Pathol* 1989, **135**, 351–357.
36. Carbone A, Gloghini A, Gruss H-J, Pinto A. CD40 ligand is constitutively expressed in a subset of T-cell lymphomas and on the microenvironmental reactive T-cells of follicular lymphomas and Hodgkin's disease. *Am J Pathol* 1995, **147**, 912–922.
37. Falini B, Bigerna B, Pasqualucci L, *et al*. Distinctive expression pattern of the BCL-6 protein in nodular lymphocyte predominance Hodgkin's disease. *Blood* 1996, **87**, 465–471.
38. Banchereau J, Bazan F, Blanchard D, *et al*. The CD40 antigen and its ligand. *Annu Rev Immunol* 1994, **12**, 881–922.
39. Carbone A, Gloghini A, Gattei V, *et al*. Expression of functional CD40 antigen on Reed-Sternberg cells and Hodgkin's diseases cell lines. *Blood* 1995, **85**, 780–789.
40. Carbone A, Gloghini A, Gruss H-J, Pinto A. CD40 antigen expression on Reed-Sternberg cells. A reliable diagnostic tool for Hodgkin's Disease. *Am J Pathol* 1995, **146**, 780–781.
41. Carbone A, Gloghini A, Pinto A. CD40: a sensitive marker of Reed-Sternberg cells. *Blood* 1996, **87**, 4918–4919.
42. Carbone A, Gloghini A, Larocca LM, *et al*. HIV-associated Hodgkin's disease derives from post-germinal center B-cells. *Blood* 1999, **93**, 2319–2326.
43. Ames ED, Conjalka MS, Goldberg AF, *et al*. Hodgkin's disease and AIDS. Twenty-three new cases and a review of the literature. *Hematol Oncol Clin North Am* 1991, **5**, 343–356.
44. Tirelli U, Errante D, Vaccher E, *et al*. Hodgkin's disease in 92 patients with HIV infection: the Italian experience. GICAT (Italian Cooperative Group on AIDS & Tumors). *Ann Oncol* 1992, **3**(Suppl. 4), 69–72.
45. Vaccher E, Spina M, Tirelli U. Clinical aspects and management of Hodgkin's disease and other tumours in HIV-infected individuals. *Eur J Cancer* 2001, **37**, 1306–1315.
46. Dolcetti R, Boiocchi M. Epstein–Barr virus in the pathogenesis of Hodgkin's disease. *Biomed Pharmacother* 1998, **52**, 13–25.
47. Flavell KJ, Murray PG. Hodgkin's disease and the Epstein–Barr virus. *J Clin Pathol: Mol Pathol* 2000, **53**, 262–269.
48. Chang KL, Albujar PF, Chen YY, Johnson RM, Weiss LM. High prevalence of Epstein–Barr virus in the Reed-Sternberg cells of Hodgkin's disease occurring in Peru. *Blood* 1993, **81**, 496–501.
49. Leoncini L, Spina D, Nyong'o A, *et al*. Neoplastic cells of Hodgkin's disease show differences in EBV expression between Kenya and Italy. *Int J Cancer* 1996, **65**, 781–784.
50. Weinreb M, Day PJ, Niggli F, *et al*. The role of Epstein–Barr virus in Hodgkin's disease from different geographical areas. *Arch Dis Child* 1996, **74**, 27–31.
51. Uccini S, Monardo F, Stoppacciaro 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.
52. Carbone A, Tirelli U, Gloghini A, Volpe R, Boiocchi M. Human immunodeficiency virus-associated systemic lymphomas may be subdivided into two main groups according to Epstein–Barr viral latent gene expression. *J Clin Oncol* 1993, **11**, 1674–1681.
53. Herndier BG, Sanchez HC, Chang KL, Chen Y-Y, Weiss LM. High prevalence of Epstein–Barr virus in the Reed-Sternberg cells of HIV-associated Hodgkin's disease. *Am J Pathol* 1993, **142**, 1073–1079.
54. Hamilton-Dutoit SJ, Pallesen G, Karkov J, Skinhoj P, Franzmann MB, Pedersen C. Identification of EBV-DNA in tumour cells of AIDS-related lymphomas by in-situ hybridisation. *Lancet* 1989, **1**, 554–558.
55. Hamilton-Dutoit SJ, Raphael M, Audouin J, *et al*. In situ demonstration of Epstein–Barr virus small RNAs (EBER 1) in acquired immunodeficiency syndrome-related lymphomas: correlation with tumor morphology and primary site. *Blood* 1993, **82**, 619–624.
56. Boiocchi M, De Re V, Gloghini A, *et al*. High incidence of monoclonal EBV episomes in Hodgkin's disease and anaplastic large-cell KI-1-positive lymphomas in HIV-1-positive patients. *Int J Cancer* 1993, **54**, 53–59.
57. Boiocchi M, Dolcetti R, De Re V, Gloghini A, Carbone A. Demonstration of a unique Epstein–Barr virus-positive cellular clone in metachronous multiple localizations of Hodgkin's disease. *Am J Pathol* 1993, **142**, 33–38.
58. Pallesen G, Sandvej K, Hamilton-Dutoit SJ, Rowe M, Young LS. Activation of Epstein–Barr virus replication in Hodgkin and Reed-Sternberg cells. *Blood* 1991, **78**, 1162–1165.
59. Brousset P, Knecht H, Rubin B, *et al*. Demonstration of Epstein–Barr virus replication in Reed-Sternberg cells of Hodgkin's disease. *Blood* 1993, **82**, 872–876.
60. 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.
61. Siebert JD, Ambinder RF, Napoli VM, Quintanilla-Martinez L, Banks PM, Gulley ML. Human immunodeficiency virus-associated Hodgkin's disease contains latent, not replicative, Epstein–Barr virus. *Hum Pathol* 1995, **26**, 1191–1195.
62. Pallesen G, Hamilton-Dutoit SJ, Rowe M, *et al*. Expression of Epstein–Barr virus replicative proteins in AIDS-related non-Hodgkin's lymphoma cells. *J Pathol* 1991, **165**, 289–299.
63. Young LS, Dawson CW, Eliopoulos AG. The expression and function of Epstein–Barr virus encoded latent genes. *J Clin Pathol: Mol Pathol* 2000, **53**, 238–247.
64. Cohen JI. Epstein–Barr virus infection. *N Engl J Med* 2000, **343**, 481–490.
65. Bellas C, Santón A, Manzanal A, *et al*. Pathological, immunological, and molecular features of Hodgkin's disease associated with HIV infection. Comparison with ordinary Hodgkin's disease. *Am J Surg Pathol* 1996, **20**, 1520–1524.

66. Gaidano G, Carbone A, Dalla-Favera R. Pathogenesis of AIDS-related lymphomas. Molecular and histogenetic heterogeneity. *Am J Pathol* 1998, **152**, 623–630.
67. Komano J, Maruo S, Kurozumi K, Oda T, Takada K. Oncogenic role of Epstein–Barr virus-encoded RNAs in Burkitt's lymphoma cell line Akata. *J Virol* 1999, **73**, 9827–9831.
68. Wilson JB, Levine AJ. The oncogenic potential of Epstein–Barr virus nuclear antigen 1 in transgenic mice. *Curr Top Microbiol Immunol* 1992, **182**, 375–384.
69. Dawson CW, Rickinson AB, Young LS. Epstein–Barr virus latent membrane protein inhibits human epithelial cell differentiation. *Nature* 1990, **344**, 777–780.
70. Fahraeus R, Rymo L, Rhim JS, Klein G. Morphological transformation of human keratinocytes expressing the LMP gene of Epstein–Barr virus. *Nature* 1990, **345**, 447–449.
71. Wang D, Liebowitz D, Kieff E. An EBV membrane protein expressed in immortalized lymphocytes transforms established rodent cells. *Cell* 1985, **43**, 831–840.
72. Kaye KM, Izumi KM, Kieff E. Epstein–Barr virus latent membrane protein 1 is essential for B-lymphocyte growth transformation. *Proc Natl Acad Sci USA* 1993, **90**, 9150–9154.
73. Moorthy RK, Thorley-Lawson DA. All three domains of the Epstein–Barr virus-encoded latent membrane protein LMP-1 are required for transformation of rat-1 fibroblasts. *J Virol* 1993, **67**, 1638–1646.
74. Zimber-Strobl U, Kempkes B, Marschall G, *et al*. Epstein–Barr virus latent membrane protein (LMP1) is not sufficient to maintain proliferation of B cells but both it and activated CD40 can prolong their survival. *EMBO J* 1996, **15**, 7070–7078.
75. Calender A, Billaud M, Aubry JP, Banchereau J, Vuillaume M, Lenoir GM. Epstein–Barr virus (EBV) induces expression of B-cell activation markers on in vitro infection of EBV-negative B-lymphoma cells. *Proc Natl Acad Sci USA* 1987, **84**, 8060–8064.
76. Wang D, Liebowitz D, Wang F, *et al*. Epstein–Barr virus latent infection membrane protein alters the human B-lymphocyte phenotype: deletion of the amino terminus abolishes activity. *J Virol* 1988, **62**, 4173–4184.
77. Henderson S, Rowe M, Gregory C, *et al*. Induction of bcl-2 expression by Epstein–Barr virus latent membrane protein 1 protects infected B cells from programmed cell death. *Cell* 1991, **65**, 1107–1115.
78. Wang S, Rowe M, Lundgren E. Expression of the Epstein–Barr virus transforming protein LMP1 causes a rapid and transient stimulation of the Bcl-2 homologue Mcl-1 levels in B-cell lines. *Cancer Res* 1996, **56**, 4610–4613.
79. Laherty CD, Hu HM, Opipari AW, Wang F, Dixit VM. The Epstein–Barr virus LMP1 gene product induces A20 zinc finger protein expression by activating nuclear factor kappa B. *J Biol Chem* 1992, **267**, 24157–24160.
80. Liebowitz D, Kopan R, Fuchs E, Sample J, Kieff E. An Epstein–Barr virus transforming protein associates with vimentin in lymphocytes. *Mol Cell Biol* 1987, **7**, 2299–2308.
81. Birkenbach M, Liebowitz D, Wang F, Sample J, Kieff E. Epstein–Barr virus latent infection membrane protein increases vimentin expression in human B-cell lines. *J Virol* 1989, **63**, 4079–4084.
82. Kim SH, Shin YK, Lee IS, *et al*. Viral latent membrane protein 1 (LMP-1)-induced CD99 down-regulation in B cells leads to the generation of cells with Hodgkin's and Reed-Sternberg phenotype. *Blood* 2000, **95**, 294–300.
83. Gires O, Zimber-Strobl U, Gonnella R, *et al*. Latent membrane protein 1 of Epstein–Barr virus mimics a constitutively active receptor molecule. *EMBO J* 1997, **16**, 6131–6140.
84. Mosialos G, Birkenbach M, Yalamanchili R, VanArsdale T, Ware C, Kieff E. The Epstein–Barr virus transforming protein LMP1 engages signaling proteins for the tumor necrosis factor receptor family. *Cell* 1995, **80**, 389–399.
85. Devergne O, Hatzivassiliou E, Izumi KM, *et al*. Association of TRAF1, TRAF2, and TRAF3 with an Epstein–Barr virus LMP1 domain important for B-lymphocyte transformation: role in NF-kappaB activation. *Mol Cell Biol* 1996, **16**, 7098–7108.
86. Izumi KM, Kieff ED. The Epstein–Barr virus oncogene product latent membrane protein 1 engages the tumor necrosis factor receptor-associated death domain protein to mediate B lymphocyte growth transformation and activate NF-kappaB. *Proc Natl Acad Sci USA* 1997, **94**, 12592–12597.
87. Izumi KM, McFarland EC, Ting AT, Riley EA, Seed B, Kieff ED. The Epstein–Barr virus oncoprotein latent membrane protein 1 engages the tumor necrosis factor receptor-associated proteins TRADD and receptor-interacting protein (RIP) but does not induce apoptosis or require RIP for NF-kappaB activation. *Mol Cell Biol* 1999, **19**, 5759–5767.
88. Mayo MW, Baldwin AS. The transcription factor NF-kappaB: control of oncogenesis and cancer therapy resistance. *Biochim Biophys Acta* 2000, **1470**, M55–62.
89. Wisdom R. AP-1: one switch for many signals. *Exp Cell Res* 1999, **253**, 180–185.
90. Gires O, Kohlhuber F, Kilger E, *et al*. Latent membrane protein 1 of Epstein–Barr virus interacts with JAK3 and activates STAT proteins. *EMBO J* 1999, **18**, 3064–3073.
91. Leonard W, O'Shea J. JAKs and STATs: biological implications. *Annu Rev Immunol* 1998, **16**, 293–322.
92. Kilger E, Kieser A, Baumann M, Hammerschmidt W. Epstein–Barr virus-mediated B-cell proliferation is dependent upon latent membrane protein 1, which simulates an activated CD40 receptor. *EMBO J* 1998, **17**, 1700–1709.
93. Nakagomi H, Dolcetti R, Bejarano MT, Pisa P, Kiessling R, Masucci MG. The Epstein–Barr virus latent membrane protein-1 (LMP1) induces interleukin-10 production in Burkitt lymphoma lines. *Int J Cancer* 1994, **57**, 240–244.
94. Herbst H, Foss HD, Samol J, *et al*. Frequent expression of interleukin-10 by Epstein–Barr virus-harboring tumor cells of Hodgkin's disease. *Blood* 1996, **87**, 2918–2929.
95. Dukers DF, Meij P, Vervoort MB, *et al*. Direct immunosuppressive effects of EBV-encoded latent membrane protein 1. *J Immunol* 2000, **165**, 663–670.
96. Frisan T, Sjoberg J, Dolcetti R, *et al*. Local suppression of Epstein–Barr virus (EBV)-specific cytotoxicity in biopsies of EBV-positive Hodgkin's disease. *Blood* 1995, **86**, 1493–1501.
97. Gratama JW, Ernberg I. Molecular epidemiology of Epstein–Barr virus infection. *Adv Cancer Res* 1995, **67**, 197–255.
98. Rickinson AB, Young LS, Rowe M. Influence of the Epstein–Barr virus nuclear antigen EBNA-2 on the growth phenotype of virus-transformed B cells. *J Virol* 1987, **61**, 1310–1317.
99. Gledhill S, Gallagher A, Jones DB, *et al*. Viral involvement in Hodgkin's disease: detection of clonal type A Epstein–Barr virus genomes in tumour samples. *Br J Cancer* 1991, **64**, 227–232.
100. Boyle MJ, Vasak E, Tschuchnigg M, *et al*. Subtypes of Epstein–Barr virus (EBV) in Hodgkin's disease: association between B-type EBV and immunocompromise. *Blood* 1993, **81**, 468–474.
101. De Re V, Boiocchi M, De Vita S, *et al*. Subtypes of Epstein–Barr virus in HIV-1-associated and HIV-1-unrelated Hodgkin's disease cases. *Int J Cancer* 1993, **54**, 895–898.
102. Lin JC, Lin SC, De BK, Chan WC, Evatt BL. Precision of genotyping of Epstein–Barr virus by polymerase chain reaction using three gene loci (EBNA-2, EBNA-3C, and EBER): predominance of type A virus associated with Hodgkin's disease. *Blood* 1993, **81**, 3372–3381.
103. Boyle MJ, Sewell WA, Sculley TB, *et al*. Subtypes of Epstein–Barr virus in human immunodeficiency virus-associated non-Hodgkin lymphoma. *Blood* 1991, **78**, 3004–3011.
104. Knecht H, Bachmann E, Brousset P, *et al*. Deletions within the LMP-1 oncogene of Epstein–Barr virus are clustered in Hodgkin's

disease and identical to those observed in nasopharyngeal carcinoma. *Blood* 1993, **82**, 2937–2942.
105. Sandvej K, Peh S-C, Andresen BS, Pallesen G. Identification of potential hot spots in the carboxy-terminal part of the Epstein–Barr virus (EBV) BNLF-1 gene in both malignant and benign EBV-associated diseases: high frequency of a 30-bp deletion in Malaysian and Danish peripheral T-cell lymphomas. *Blood* 1994, **84**, 4053–4060.
106. Santón A, Manzanal AI, Campo E, Bellas C. Deletions in the Epstein–Barr virus latent membrane protein-1 oncogene in Hodgkin's disease. *J Clin Pathol: Mol Pathol* 1995, **48**, M184–187.
107. Dolcetti R, Zancai P, De Re V, *et al*. Epstein–Barr virus strains with latent membrane protein-1 deletions: prevalence in the Italian population and high association with human immunodeficiency virus-related Hodgkin's disease. *Blood* 1997, **89**, 1723–1731.
108. Hu LF, Zabarovsky ER, Chen F, *et al*. Isolation and sequencing of the Epstein–Barr virus BNLF-1 gene (LMP-1) from a Chinese nasopharyngeal carcinoma. *J Gen Virol* 1991, **72**, 2399–2409.
109. Chen ML, Tsai CN, Liang CL, *et al*. Cloning and characterization of the latent membrane protein (LMP) of a specific Epstein–Barr virus variant derived from the nasopharyngeal carcinoma in the Taiwanese population. *Oncogene* 1992, **7**, 2131–2140.
110. Hu LF, Chen F, Zheng X, *et al*. Clonability and tumorigenicity of human epithelial cells expressing the EBV encoded membrane protein LMP1. *Oncogene* 1993, **8**, 1575–1583.
111. Trivedi P, Hu LF, Chen F, *et al*. Epstein–Barr virus (EBV)-encoded membrane protein LMP1 from a naso pharyngeal carcinoma is non-immunogenic in a murine model system, in contrast to a B cell-derived homologue. *Eur J Cancer* 1994, **30A**, 84–88.
112. Rothenberger S, Bachmann S, Knecht H. Molecular and functional analysis of the Epstein–Barr virus LMP-1 oncogene promoter in lymphoproliferative diseases. *Exp Hematol* 1997, **25**, 1326–1332.
113. Moorthy RK, Thorley-Lawson DA. All three domains of the Epstein–Barr virus-encoded latent membrane protein LMP-1 are required for transformation of Rat-1 fibroblasts. *J Virol* 1993, **67**, 1638–1646.
114. Khanim F, Yao QY, Niedobitek G, Sihota S, Rickinson AB, Young LS. Analysis of Epstein–Barr virus gene polymorphisms in normal donors and in virus-associated tumors from different geographic locations. *Blood* 1996, **88**, 3491–3501.
115. Carbone A, Gaidano G, Gloghini A, *et al*. Differential expression of BCL-6, CD138/syndecan-1 and EBV-encoded latent membrane protein-1 identifies distinct histogenetic subsets of acquired immunodeficiency syndrome-related non-Hodgkin's lymphomas. *Blood* 1998, **91**, 747–755.
116. Carbone A, Gloghini A, Gattei V, *et al*. Reed-Sternberg cells of classical Hodgkin's disease react with the plasma cell specific monoclonal antibody B-B4 and express human syndecan-1. *Blood* 1997, **89**, 3787–3794.
117. Carbone A, Gloghini A, Gaidano G, *et al*. Expression status of BCL-6 and syndecan-1 identifies distinct histogenetic subtypes of Hodgkin's disease. *Blood* 1998, **92**, 2220–2228.
118. Cattoretti G, Zhang J, Cleary AM, *et al*. Downregulation of BCL-6 gene expression by CD40 and EBV latent membrane protein-1 (LMP-1) and its block in lymphoma carrying BCL-6 rearrangements. *Blood* 1997, **90**, 175a (abstr).
119. Iida S, Rao PH, Butler M, *et al*. Deregulation of MUM1/IRF4 by chromosomal translocation in multiple myeloma. *Nat Genet* 1997, **17**, 226–230.
120. Carbone A, Gloghini A, Larocca ML, *et al*. Expression profile of MUM1/IRF4, BCL-6, and CD138/syndecan-1 defines novel histogenetic subsets of human immunodeficiency virus-related lymphomas. *Blood* 2001, in press.
121. Doyle TJ, Venkatachalam KK, Maeda K, Saeed SM, Tilchen E. Hodgkin's disease in renal transplant recipients. *Cancer* 1983, **51**, 245–247.
122. Oldhafer KJ, Bunzendahl H, Frei U, Kemnitz J, Vogt P, Pichlmayr R. Primary Hodgkin's lymphoma: an unusual cause of graft dysfunction after kidney transplantation. *Am J Med* 1989, **87**, 218–220.
123. Jaccard A, Emilie JF, Metivier F, Clauvel JP, d'Agay MF. Epstein–Barr virus associated Hodgkin's disease following renal transplantation. *Am J Hematol* 1994, **46**, 256–267.
124. Rowlings PA, Curtis RE, Passweg JR, *et al*. Increased incidence of Hodgkin's disease after allogeneic bone marrow transplantation. *J Clin Oncol* 1999, **17**, 3122–3127.
125. Ferraccioli GF, De Vita S, Casatta L, *et al*. EBV associated Hodgkin's lymphoma in a rheumatoid patient treated with methotrexate and cyclosporin A. *Arthritis Rheum*. 1995, **38**, 867-868
126. Kamel OW, Weiss LM, van de Rijn M, Colby TV, Kingma DW, Jaffe ES. Hodgkin's disease and lymphoproliferations resembling Hodgkin's disease in patients receiving long-term low-dose methotrexate therapy. *Am J Surg Pathol* 1996, **20**, 1279–1287.
127. Tinguely M, Vonlanthen R, Muller E, *et al*. Hodgkin's disease-like lymphoproliferative disorders in patients with different underlying immunodeficiency states. *Mod Pathol* 1998, **11**, 307–312.