The Molecular Pathogenesis of Hodgkin Lymphoma

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Review Article

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Abstract

Hodgkin lymphoma (HL) is an unusual malignancy in that the tumour cells, the Hodgkin and Reed-Sternberg (HRS) cells, are a minor component of the tumour mass, the bulk of which is a mixed cellular infiltrate. There is compelling evidence that HRS cells are clonal B-cells that have lost their B-cell phenotype. Mature B-cells lacking B-cell receptors would normally die by apoptosis and therefore HRS cells must have developed mechanisms to facilitate survival. The escape from apoptosis and transcriptional reprogramming of HRS cells are interlinked and appear central to disease pathogenesis. The Epstein-Barr virus (EBV) is present in the HRS cells of a proportion of cases and expresses genes with a plausible oncogenic function. It is likely that EBV plays a role in reprogramming and survival through dysregulation of several signalling networks and transcription factors, including NF-κB. Activation of NF-κB is a feature of all HRS cells and gene mutations affecting this pathway appear common in EBV-negative HL. The HRS cell furthers its own survival by attracting a supportive microenvironment of immune and stromal cells, and suppressing local immune responsiveness. Although many questions remain unanswered, the last two decades have witnessed a considerable increase in our knowledge of this complex disease.

Word count: 200

Body of Text

Introduction

Hodgkin lymphoma (HL) is one of the commonest lymphomas in the developed world, with an incidence of approximately 3 per 100,000 person years.[1] First described in 1832 by Thomas Hodgkin[2] and subsequently called Hodgkin's disease, it is now recognised as a clonal B-cell neoplasm and has therefore been renamed Hodgkin lymphoma. HL comprises two entities, nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) and classical Hodgkin lymphoma (cHL.) A striking feature of both entities is that the malignant cell is rare in the involved tissue, accounting for only around 1% of the tumour mass.[3] The remainder of the tumour comprises a cellular infiltrate with an admixture of different cell types. Despite these similarities, the clinical features, cell of origin and molecular pathogenesis of NLPHL and cHL are distinct and they are best regarded as separate diseases. In NLPHL, the malignant cell is the LP cell (formerly called L&H cell) or "popcorn" cell, so-named because of its characteristic multilobated morphology. cHL is characterised by the presence of the Reed-Sternberg cell, a large bi-or multinucleate cell, and its mononuclear equivalent, the Hodgkin cell, which together are referred to as HRS cells. cHL is further divided into nodular sclerosing (NS), mixed cellularity (MC), lymphocyte rich classical (LR) and lymphocyte-depleted (LD) subtypes on the basis of the morphology of the HRS cells and the composition of the background cellular infiltrate. Approximately one-third of cases of cHL in the developed world are associated with Epstein-Barr Virus (EBV), where the virus is believed to play a causal role.[4] cHL accounts for 95% of cases and will be the focus of this review.

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Cell of Origin

The cellular origin of the tumour cells in HL remained elusive for many years but microdissection coupled with analysis of immunoglobulin (Ig) genes revealed that these cells are usually clonal B-cells. HRS cells, from nearly all cHL cases, and LP cells have detectable rearrangements of Ig heavy and/or light chain genes, confirming a B-cell origin[5,6] and, in any given case, the rearrangements are identical, proving the clonal nature of the disease.[5,7,8] Furthermore, the Ig variable (IgV) gene regions show evidence of somatic hypermutation, revealing a germinal centre (GC) or post-GC origin.[9] The cHL and B-cell non-Hodgkin lymphoma (B-NHL) components of composite lymphomas generally harbour identical IgV gene rearrangements but have distinct somatic Ig gene mutations. This strongly suggests that both have arisen from a common precursor which is a pre-GC or GC B-cell.[10] In LP cells intraclonal IgV gene diversity is observed indicating on-going somatic hypermutation whereas HRS cells show identical somatic hypermutations consistent with a later stage of B-cell differentiation.[5,7,8] "Crippling" mutations, resulting in non-functional Ig genes, are observed in around 25% of cHL cases.[5,7,10,11] Rarely (<2%) HRS cells harbour rearranged T-cell receptor genes suggesting a T-cell origin in a small minority of cases.[12-14]

Comment [kf1]: This change addresses part of point 3 in that it explains the evolution of the HRS cell in relation to B cell and B-NHL

The phenotype of the HRS cell does not reveal its B-cell origin. Markers of B-lineage, such as CD20, CD19, CD79 and surface Ig, and the transcription factors OCT2, BOB1 and PU1 are generally down-regulated in the HRS cell.[15,16] Expression of the B-cell-specific transcription factor PAX5 is usually retained, albeit at low levels, and can be helpful in distinguishing cHL from T-cell lymphomas.[17] HRS cells classically express the tumour-necrosis factor-

receptor (TNFR) family member CD30 and the myeloid marker CD15.

Aberrant expression by the HRS cell of T-cell markers such as CD3, CD4 and

granzyme B[18], and dendritic cell markers such as fascin and TARC (Thymus

activation regulated chemokine, also known as CCL17) is observed.[19] In

contrast, LP cells express B-cell markers including CD20, CD79, PAX5, OCT2

and BOB1.

Normally, GC B-cells which lack a functional B-cell receptor (BCR) complex

would undergo apoptosis. The survival of the HRS cell in the face of apoptotic

triggers is therefore considered central to cHL oncogenesis. Mutation or viral

infection may counter apoptosis; alternatively, transcriptional reprogramming

with loss of the B-cell signature may abrogate the intrinsic requirement for

tonic BCR signalling.

Reprogramming of HRS cells

Although HRS cells in some cases have crippling mutations of Ig genes

leading to lack of functional BCRs, it is clear that epigenetic modification plays

an important role in transcriptional down-regulation of B-lineage-specific

genes. The simultaneous silencing of a group of genes by promoter

methylation suggests that a master transcriptional regulator(s) may be

involved.[20,21] Activation of Notch1 signalling and inhibition of the transcription

factors E2A and EBF, appear important in this regard. Notch1 promotes

transcription of T-cell genes and suppresses the B-cell programme of

differentiation through promoting the degradation of E2A [22] and blocking the

DNA binding of EBF.[23] E2A function is also inhibited by over-expression of

activated B-cell factor 1 (ABF1) and inhibitor of differentiation and DNA

binding 2 (ID2.)[24-26] In addition, activation of Notch1 inhibits PAX5 both at

transcriptional and post-translational levels.[27] Notch1 up-regulation is caused

in part by stimulation by Jagged1, which is produced by the reactive cells

surrounding the HRS cell. [28,29]

Survival and proliferation of HRS cells

Nuclear factor kappa B (NF-κB) is a family of transcription factors which play

a key role in numerous cellular responses including inflammatory responses

and cell fate decisions.[30] This protein family includes five members, RelA

(p65), RelB, c-Rel, NF-κB1 (p50 and its precursor p105) and NF-κB2 (p52

and its precursor p100), which function as homodimers and heterodimers.[31]

In the absence of stimulation, NF-κB is maintained in an inactive state, mostly

in the cytoplasm, by binding the inhibitory proteins IκBα, IκBβ, IκBε, and the

precursor proteins p105 and p100. NF-κB is activated following ligation of

cellular receptors, including the tumour necrosis factor (TNF) receptor

superfamily, resulting in IκB-kinase (IKK) activation and proteolytic

degradation of IκBs. The NF-κB complex is then free to bind its DNA target.

Activation of NF-κB is normally transient and tightly controlled; however, in

HRS cells NF-κB is constitutively active.[32,33]

NF-κB activation in HRS cells may be explained by a number of different

mechanisms. First, many TNF receptors are over-expressed by HRS cells,

including CD30, CD40, RANK and CD95.[34,35] In addition, the cellular milieu in

which the HRS cell resides produces ligands of these receptors, leading to

paracrine stimulation. Moreover, intrinsic over-expression of CD30 by the

HRS cell has been suggested to contribute to NF-κB activation in the HRS

cell.[36] EBV can directly contribute to activation of NF-κB though its protein

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LMP-1 which mimics CD40 signalling. Secondly, deleterious mutations of the genes encoding IκB proteins, in particular IκBα, have been described in 10-20% of cases of cHL.[37-41] Thirdly, amplification of the chromosomal region including the *c-Rel* gene has been reported in nearly 50% of cases of cHL cells.[42-44]

More recently, inactivating mutations of the *TNFα-induced protein 3* (*TNFAIP3*) gene, which encodes the protein A20, have been described in cHL.[45,46] A20 is a deubiquitinase and ubiquitinase which negatively regulates NF-κB and prevents excessive or prolonged activation.[45] Schmitz et al (2010)[46] using cHL-derived cell lines and single microdissected HRS cells, demonstrated that A20 was mutated or deleted in a clonal, somatic fashion in 16 of 36 primary cases. In most cases, the mutation was bi-allelic and reconstitution of A20 activity reduced NF-κB transcriptional activity and led to cytotoxicity. Kato and colleagues (2010)[45] verified the importance of this gene in a genome-wide analysis of genetic lesions in lymphoma, where pooled microdissected cells from 5 of 24 primary cases of cHL demonstrated a mutation or deletion in this gene. All clearly inactivating mutations were found in EBV-negative cHL cases, suggesting that EBV infection and A20 mutations are alternative ways of providing sustained NF-κB activation signal in HRS cells.[46]

Activation and dysregulation of the JAK-STAT pathway have also been implicated in the proliferation and apoptosis resistance of HRS cells. JAK-STAT signalling is one of the central mechanisms of signalling by cytokines including IL-5, IL-6, IL-9, IL-13 and GM-CSF.[47] In cHL, cytokines which can

initiate JAK-STAT activation are produced in abundance, and phosphorylated STAT3, STAT5 and STAT6 are present at high levels in the nuclei of HRS cells.,[48-52] Genomic lesions affecting the JAK-STAT pathway have also been demonstrated with copy number amplifications of the *JAK2* gene (9p24)[53] and mutations of *SOCS-1*, a negative regulator of JAK.[54] Several other signalling pathways have been shown to be deregulated in HL, including PI3K-Akt-mTOR,[55] MAPK-MEK-ERK[56] and the AP1-JUN/Fos pathway whose targets include CD30 and Galectin-1.[57,58]

Small non-coding RNAs (microRNAs) are now recognised to be important in cancer development and it is clear that they are differentially expressed in HL. miR-155 is expressed at very high levels in HL cell lines and primary tissue[59] where it may be targeting the B-cell transcription factor PU.1 for down-regulation. MicroRNA expression profiling of microdissected HRS cells demonstrated the upregulation of miRs 9, 16, 18a, 20a, 21,30b, 30a-5p, 140, 155, 186, 196a, & 374, and the downregulation of miRs 200, 520a & 614.[60] Analysis of whole cHL tumours produced different results, probably reflecting alterations of microRNA profile in the infiltrating cells.[61] Whilst putative targets for these microRNAs, such as *BCL2*, *HOXA7* and *PTEN*, would imply a role in the survival, proliferation and reprogramming of HRS cells, functional studies to prove these associations have not been performed. Ongoing studies are likely to elucidate a more precise role for microRNAs in the transcriptional regulation of the HRS cell.

Thus, multiple transcriptional and signalling pathways are disrupted in HL, and are thought to co-operate to increase HRS cell proliferation, reduce apoptosis

and promote a favourable cellular environment through the release of multiple cytokines and chemokines (see below.)

Chromosomal genetic abnormalities in the HRS cell

Unlike many B-NHLs where single recurrent cytogenetic abnormalities are a feature, no single pathognomonic cytogenetic abnormality has been demonstrated in HL. Conventional comparative genomic hybridisation (CGH) studies have demonstrated numerous chromosomal imbalances including recurrent gains of 2p (including the *REL* oncogene), 9p (including *JAK2*), 16p, 17p, 17q and 22q and loss of 13q.[53,62,63]A recent array-based CGH analysis[64] found gains of 2p, 9p, 12p, 16p, 17p, 17q, 19p, 19q, 20q, 21q and losses of 1p, 6q,7q, 8p, 11q, and 13q. Minimally gained and lost regions were defined, including regions harbouring genes involved in NFκB signalling such as *REL* (2p)*, IKBKB, CD40, MAP3K14* and *TNFAIP3.* An association between cytogenetic findings and clinical outcome was also demonstrated, with patients who had gains of 16p11.2-13.3, which harbours the multi-drug resistance gene ABCC1, having poorer disease-specific survival.

Multiple cytogenetic abnormalities have been demonstrated by FISH and FICTION, including breakpoints at 7q22, 7q32, 11q23, 13p11 and 14q32.[65] A proportion of NLPHL and cHL cases have been shown to have translocations involving the IgH gene (14q32), as seen in various B-NHLs. *BCL6* has been found to be a translocation partner in NLPHL, but only infrequently in cHL.[66,67] Other genes commonly associated with Ig translocations in B-NHL, including *cyclin D1*, *BCL2* and *MYC* are not implicated in HL, where the partner genes are yet to be discovered.

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Biological overlap with B-NHL

Some types of B-NHL share morphological features and immunophenotype with cHL, hence the occasional diagnostic difficulty. These include the anaplastic variant of diffuse large B-cell lymphoma (DLBCL),[68] primary mediastinal B-cell lymphoma (PMBCL),[69] and "grey zone lymphoma", which has features intermediate between DLBCL and cHL.[70] Gene expression profiling has identified PMBCL as being more closely related to cHL than DLBCL.[71-73] It has been shown that some of the key players involved in the pathogenesis of cHL, such as *REL*[74,75] and JAK-STAT[76,77], are also important in these "intermediate" B-NHLs. It is likely that these lymphomas represent a true biological overlap with cHL, arising from a putative thymic B-cell and sharing some clinical features.

Comment [kf2]: This paragraph directly addresses point 1 & point 2. Other prognostic features related to this are not discussed as they remain controversial, and other better established prognostic markers have been omitted form the rest of the review due word limit restricting scope for fuller discussion. The references guide the reader to further information if sought.

A Hodgkin lymphoma stem cell?

Stem cells are defined by their facility for unlimited self-renewal, and their capacity to produce progeny of multiple different lineages. Leukaemic stem cells, subpopulations with the capacity for unlimited self-renewal, have been well-described. However, lymphomas, including HL arise from mature lymphocytes and the existence of a 'lymphoma stem cell' or 'lymphoma-originating cell' has yet to be proven and remains controversial. A recent study, using flow cytometry to detect the stem-cell marker aldehyde dehydrogenase, demonstrated rare populations of small cells within the cHL-derived cell lines L428 and KM-H2, which were capable of generating the predominant HRS cells.[78] Shafer and colleagues (2010) [79] recently demonstrated the presence of side-population cells, associated with a "stem" or progenitor cell phenotype, in both HL cell lines and primary tumour

specimens. Much further work remains to be done to characterise these cells

and determine whether they are clonogenic as well as clonotypic.

Interaction with the cellular microenvironment

Almost all of the cells present in HL tumour tissue comprise a reactive

infiltrate including T-cells, B-cells, eosinophils, fibroblasts, macrophages, mast

cells and plasma cells.[80] This infiltrate may be, in part, a response to the

tumour; however, it is ineffective (see below) and there is evidence that HRS

and LP cells actively recruit these infiltrating cells to the tumour, where the

paracrine signals they provide promote survival of the tumour cells. The

interaction between the tumour cells and the infiltrating cells is therefore a

critical feature of HL pathogenesis.

The infiltrating cells are largely CD4+ T-cells and those in close proximity to

the HRS cells, often forming so-called rosettes, generally demonstrate a TH2

phenotype; CD8+ cytotoxic T-lymphocytes (CTLs), TH1 CD4+ T-helper cells

and natural killer (NK) cells are notable by their absence. It is thought that

these TH2 cells are attracted by chemokines produced by the HRS cell,

including TARC[81],CCL5 (RANTES) and CCL22.[82] They express CD40 ligand

which may stimulate CD40 expressed on the surface of HRS cells, triggering

signalling cascades mentioned above. CD4+CD25+FOXP3+ regulatory T-

cells (Tregs) are also abundant among the infiltrating cells [83] Tregs suppress

tumour-antigen specific CTLs and NK cells, and may therefore protect the

tumour cell from immune attack, particularly in the case of EBV-associated

disease where viral antigens are expressed and would be expected to trigger

an immune response.[84,85] Secretion of galectin1 [58,86] and chemokines

including IL-10, TGF-β,[83] TARC, CCL5, CCL20 and CCL22.[82,87] by the HRS

Comment [KF3]: This is added to address part of point 3. It is not currently thought that the B-cells forming part of the cellular infiltrate in cHL are clonally related to the HRS cell

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cell is likely to lead to recruitment of Tregs to the tumour. In addition, mRNA expression studies suggest that TH17 cells are present in the infiltrating cells.[88]

HRS cells and fibroblasts secrete eotaxin, IL5, IL9, CCL5, CCL11 and CCL28 which may act to recruit eosinophils, which are frequently present in HL tissue.[89] The eosinophils secrete TGFβ, and express CD30 ligand which stimulates CD30 expressed on the tumour cell surface[90]. Mast cells present in the infiltrate also express CD30 ligand, and in addition may contribute to angiogenesis.[91] Other cytokines involved in the pathogenesis of HL include IL-13, which is produced by HRS cells in some cases and, since the IL-13 receptor is also expressed by HRS cells, may function in an autocrine manner to promote HRS cell survival.[92,93]

Gene expression studies have demonstrated that the genes most highly up-regulated in HL include those encoding key cytokines such as *TARC* and *Galectin 1*, thus confirming their importance in disease pathogenesis [62,94] Proteomic analysis of cell lines and primary tissue has also revealed elevated levels of TARC, IL1R2, MIF, CD26, CD44, and cathepsin S.[95] A summary of these, and other, molecules implicated in the cross-talk between HRS cells and their micro-environment is given in Table 1[24,34,36,58,81,86,87,90,91,93,96,96-116]

Epstein-Barr virus and Hodgkin lymphoma
Epstein-Barr virus (EBV) is a lymphotropic γ-herpesvirus which infects more than 95 % of the world's population. Primary infection generally occurs in childhood and is asymptomatic or subclinical but late exposure can led to the development of infectious mononucleosis (IM). Following primary infection,

the virus remains latent in memory B-cells for the lifetime of the host.[117] and is

kept in check by the cytotoxic T-lymphocyte (CTL) response.[118] Although viral

infection is usually asymptomatic, EBV is associated with a number of

cancers including cHL and some B-NHLs.

A proportion of cases of HL are associated with EBV, where the virus is

believed to play a causal role.[4] In EBV-associated tumours, EBV is detected

in all of the HRS cells (see Figure 1) and the viral infection is clonal, proving

that infection occurred prior to transformation and supporting a causative

role.[119-121]. Furthermore, HRS cells express EBV proteins including EBNA-1,

LMP-1, LMP-2 antigens and the EBER and BART RNAs – a pattern referred

to as "latency II" [117] EBNA-1 and LMP-1 are essential for transformation of B-

cells by EBV and recent data suggest that LMP-2 plays a critical role in B-cell

survival.[122] EBNA-1 is essential for maintaining the viral genome as an

episome and ensuring genome partitioning during mitosis. In addition, EBNA-

1 may support the development of the tumour through up-regulating CCL22,

and thus attracting Tregs.[98] LMP-1 can mimic the signal provided by CD40,

thus providing a means of constitutively activating NF-κB. LMP-1 is also

capable of activating p38, PI3K, AP1 and JAK-STAT signalling.[123] LMP-2 is

thought to play an important role in B-cell survival and, in vitro, is essential for

the rescue and transformation by EBV of GC B-cells lacking BCRs.[124-126]

LMP-1 and LMP-2 may also contribute to the reprogramming of the B-cell

phenotype that occurs in HRS cells;[127,128] in particular, LMP-2 constitutively

activates the Notch pathway leading to alteration of levels of the transcription

factors E2A and EBF (see above). The non-coding EBER RNAs have been

shown to suppress p21cip/waf transcription, thereby increasing apoptosis

resistance through down-regulation of p53, EGR1 and STAT1.[129] The BARTs are a group of alternatively spliced RNAs derived from the Bam*HI* A fragment of the EBV genome, which encode a large number of microRNAs. At present, their function is poorly understood but it would seem likely that they have a role in viral pathogenesis.

Although the morphology, phenotype and gene expression profile of EBV-associated and non-associated cases of cHL appear similar, there is increasing evidence that the molecular pathogenesis of these two groups of cases is distinct. First, mutations of genes encoding inhibitors of NF-κB, in particular *TNFAIP3* (A20), are more common in EBV-negative cases suggesting that these mutations substitute for LMP-1 expression [46]. Secondly, crippling mutations of Ig genes appear almost exclusive to EBV-associated cases indicating that EBV is required to rescue cells harbouring these mutations from apoptosis. Thirdly, expression of multiple receptor tyrosine kinase pathways[130] is more frequent in non-EBV-associated cases, suggesting that EBV is replacing vital oncogenic signals.

Epidemiological data also support a role for EBV in HL pathogenesis. The proportion of EBV-associated cHL cases is significantly higher in cases occurring in early childhood and older adult age groups (aged >50 years) compared to younger adults, in developing countries compared to industrialised countries, and in males compared to females.,[131,132] Cases of mixed-cellularity subtype are also significantly more likely to be EBV-associated than nodular sclerosis cases.

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There is an increased risk of EBV-associated HL following infectious

mononucleosis,[133,134] which decreases with increasing time from the illness.

The frequency of circulating EBV-infected cells is also significantly higher in

pre-treatment blood samples from EBV-associated cases when compared

with non-EBV-associated cases,[135] and patients with HL also have been

shown to have higher titres of anti-EBV antibody both at time of diagnosis and

several years prior.[136] Collectively, these data suggest that control of EBV

infection is related to risk of developing EBV-associated HL.

The immune response and cHL

An association between cHL, particularly EBV-associated disease, and

immunosuppression has been recognised for many years. Patients with

HIV/AIDS are at increased risk and, in this context, the disease is almost

always EBV-positive. EBV-associated HL is also seen following allogeneic

bone marrow transplant and solid organ-transplantation.[137] More subtle

immune suppression may also lie behind development of cHL. For instance,

reactivation of varicella zoster virus (VZV), a surrogate of immune

suppression, is a risk factor for development of HL.[138,139] Patients with EBV-

associated HL were more likely to have reactivated VZV in the year prior to

diagnosis than patients with EBV-negative disease.[140] Such subtle immune

suppression, possibly occurring as part of immune senescence, may account

for the greater predominance of EBV-associated cHL in older adults, and may

suggest an aetiological overlap with EBV-positive DLBCL of the elderly.[141]

Comment [kf4]: This addition addresses point 4.

Recent data have shown a strong association between EBV-associated HL

and HLA class I genotype suggesting that HLA-restricted CTL responses play

a key role in determining risk of EBV-associated HL. Increased risk was

associated with HLA-A*01 and decreased risk with HLA-A*02. The effects of A*01 and A*02 were independent of each other but dependent on allele copy number, such that HLA-A*01 homozygotes had an almost 10-fold greater odds of EBV-associated HL than HLA-A*02 homozygotes.[142] Whereas CTL responses to many HLA-A*02-restricted EBV epitopes have been described, there are no confirmed HLA-A*01-restricted responses to epitopes derived from either lytic or latent viral proteins.[118,143] This raises the suspicion that the increased risk of EBV-associated cHL is related to a weak EBV-specific CTL response. A weak response could influence disease risk in two ways: first, it could allow expansion of EBV-infected tumour cells; and secondly, a sub-optimal response to primary and persistent EBV infection could lead to a higher level of EBV and an increased risk of transformation.

Conclusions

The molecular pathogenesis of HL is complex; however, this remarkable disease is beginning to give up its secrets. There is good evidence that HL is a clonal B-cell neoplasm and that the global suppression of the B-cell signature results from transcriptional reprogramming. EBV is thought to play a critical role in the pathogenesis of a proportion of cases of HL, and EBV gene products appear to contribute to HRS cell survival, proliferation and reprogramming. Multiple transcription factors and signalling pathways are dysregulated in the HRS cell, most notably the NF-κB pathway. EBV infection most likely leads to NF-κB activation in EBV-associated cases and mutations of genes encoding inhibitors and regulators of NF-κB have been detected in a large proportion of EBV-negative cases. Crosstalk between the HRS cell and the reactive component of tumours also appears important in the survival and

proliferation of HRS cells and immune evasion. However, many questions

remain: is there a 'HL-initiating' cell and what is the phenotype of this cell; is

there another virus present in EBV-negative cases and does this explain the

unusual epidemiology of these cases; what are the targets of the viral and

non-viral miRNAs expressed by HRS cells; how do host factors, such as HLA

genotype, contribute to disease risk? It is hoped that a better understanding

of these issues can be exploited for the benefit of patients, with less-toxic,

more-effective targeted therapies and potentially preventative measures

Word Count: 3681

Comment [kf5]: With changes, word count is now 3681

Disclosure

The authors declare no conflict of interest.

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Acknowledgements

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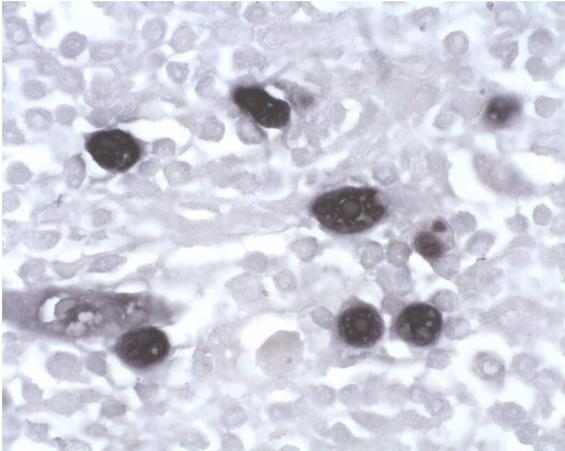
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Table 1: Cytokines, chemokines, receptors and ligands altered in classical
Hodgkin lymphoma[144,145]

Function	Cytokine/ Chemokine/ Receptor/ Ligand	Reference
Promote Th2 Response	TARC/CCL17	81
	MDC/CCL22	96,97
	CCL-20	98
	MIG	96,99
	IP-10	96
	IL-13	93
	GATA-3	24,100
	CCR-4	101
Suppress Th1 Response	IL-10	91
	TGF-β	102,103
	PD-1L	104
	Galectin-1	58,86
Promote influx of eosinophils	IL-5	105
	IL-9	106
	Eotaxin (produced by fibroblasts)	107
	CCL28	108
Promote influx of mast cells	IL-9	106
	RANTES/CCL5 (also monocytes, T-cells & eosinophils)	87
Promote influx of plasma cells	IL-6	109
Promote influx of neutrophils	IL-8	110
	GM-CSF	111
Promote influx of fibroblasts	IL-13	93
	TGF-β	102,103
	TNF-α	112,113
	MMP	114
	TIMP1 & 2	115
Autocrine growth factor	IL-13/IL-13-R (via STAT6 activation)	93
	TIMP-1	115
	IL-6	109
Activates NF-κB	CD40/CD40-R	116
	CD30/CD30-R	36,90
	TNF-α-	112,113
	RANK/RANK-L	34

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Figure 1: EBV EBER in situ hybridisation showing positive staining in the nuclei of all the tumour cells. x1000

Table 1: Cytokines, chemokines, receptors and ligands altered in classical
Hodgkin lymphoma[144,145]

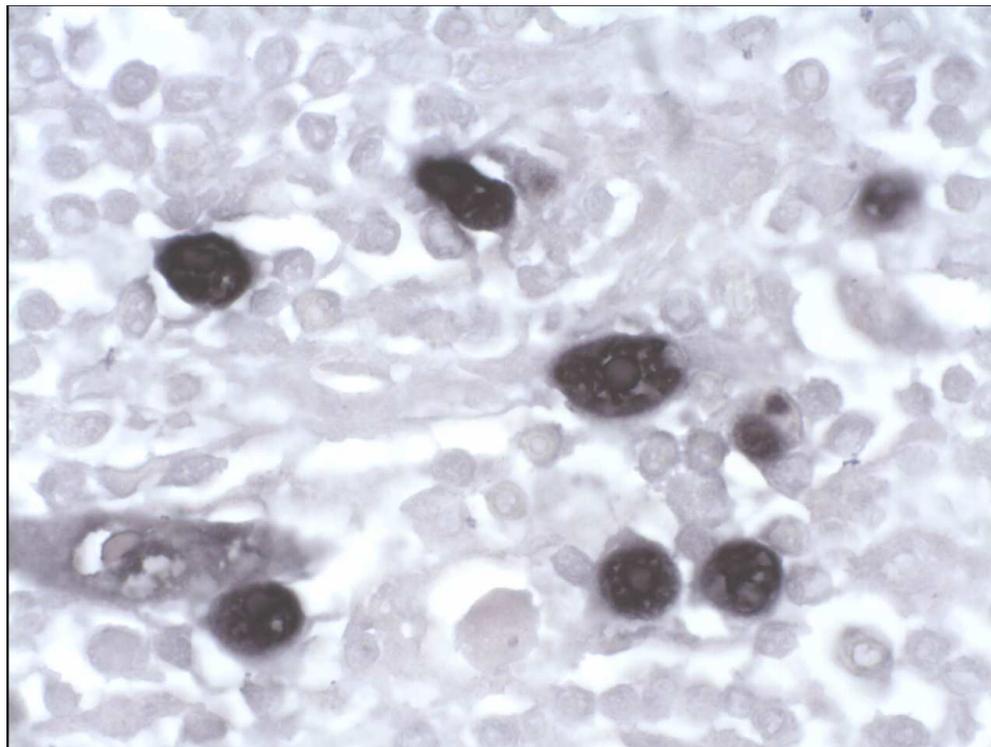| Function	Cytokine/ Chemokine/ Receptor/ Ligand	Reference
Promote Th2 Response	TARC/CCL17	81
	MDC/CCL22	96,97
	CCL-20	98
	MIG	96,99
	IP-10	96
	IL-13	93
	GATA-3	24,100
	CCR-4	101
Suppress Th1 Response	IL-10	91
	TGF-β	102,103
	PD-1L	104
	Galectin-1	58,86
Promote influx of eosinophils	IL-5	105
	IL-9	106
	Eotaxin (produced by fibroblasts)	107
	CCL28	108
Promote influx of mast cells	IL-9	106
	RANTES/CCL5 (also monocytes, T-cells & eosinophils)	87
Promote influx of plasma cells	IL-6	109
Promote influx of neutrophils	IL-8	110
	GM-CSF	111
Promote influx of fibroblasts	IL-13	93
	TGF-β	102,103
	TNF-α	112,113
	MMP	114
	TIMP1 & 2	115
Autocrine growth factor	IL-13/IL-13-R (via STAT6 activation)	93
	TIMP-1	115
	IL-6	109
Activates NF-κB	CD40/CD40-R	116
	CD30/CD30-R	36,90
	TNF-α-	112,113
	RANK/RANK-L	34

Figure 1: EBV EBER in situ hybridisation showing positive staining in the nuclei of all the tumour cells. x1000
216x162mm (150 x 150 DPI)