



**HAL**  
open science

# The Molecular Pathogenesis of Hodgkin Lymphoma

Katrina Farrell, Ruth F Jarrett

► **To cite this version:**

Katrina Farrell, Ruth F Jarrett. The Molecular Pathogenesis of Hodgkin Lymphoma. *Histopathology*, 2011, 58 (1), pp.15. <10.1111/j.1365-2559.2010.03705.x>. <hal-00610741>

**HAL Id: hal-00610741**

**<https://hal.science/hal-00610741v1>**

Submitted on 24 Jul 2011

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization



**The Molecular Pathogenesis of Hodgkin Lymphoma**

Journal:	<i>Histopathology</i>
Manuscript ID:	HISTOP-08-10-0462.R1
Wiley - Manuscript type:	Review
Date Submitted by the Author:	27-Sep-2010
Complete List of Authors:	Farrell, Katrina; University of Glasgow, LRF Virus Centre Jarrett, Ruth; University of Glasgow, LRF Virus Centre
Keywords:	Hodgkin Disease, Reed-Sternberg Cells, Epstein-Barr Virus Infections, Transcription Factors, B-lymphocytes

SCHOLARONE™  
Manuscripts

view

Review Article

## **The Molecular Pathogenesis of Hodgkin Lymphoma**

### **Authors**

Katrina Farrell, Ruth F. Jarrett

### **Address for correspondence**

LRF Virus Centre

Botham Building

University of Glasgow

Bearsden Road

Glasgow

G61 1QH

Tel: 0141 330 5707

E-mail: [katrina.farrell@glasgow.ac.uk](mailto:katrina.farrell@glasgow.ac.uk)

### **Keywords**

Hodgkin Disease, Reed-Sternberg Cells, Epstein-Barr Virus Infections,

Transcription Factors, B-lymphocytes

**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- $\kappa$ B. Activation of NF- $\kappa$ B 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.<sup>1</sup> First described in 1832 by Thomas Hodgkin<sup>2</sup> 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.<sup>3</sup> 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.<sup>4</sup> cHL accounts for 95% of cases and will be the focus of this review.

## 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<sup>5,6</sup> and, in any given case, the rearrangements are identical, proving the clonal nature of the disease.<sup>5,7,8</sup> Furthermore, the Ig variable (IgV) gene regions show evidence of somatic hypermutation, revealing a germinal centre (GC) or post-GC origin.<sup>9</sup> 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.<sup>10</sup> 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.<sup>5,7,8</sup> “Crippling” mutations, resulting in non-functional Ig genes, are observed in around 25% of cHL cases.<sup>5,7,10,11</sup> Rarely (<2%) HRS cells harbour rearranged T-cell receptor genes suggesting a T-cell origin in a small minority of cases.<sup>12-14</sup>

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.<sup>15,16</sup> 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.<sup>17</sup> HRS cells classically express the tumour-necrosis factor-



**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

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<sup>18</sup>, and dendritic cell markers such as fascin and TARC (Thymus activation regulated chemokine, also known as CCL17) is observed.<sup>19</sup> 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.<sup>20,21</sup> 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<sup>22</sup> and blocking the DNA binding of EBF.<sup>23</sup> E2A function is also inhibited by over-expression of activated B-cell factor 1 (ABF1) and inhibitor of differentiation and DNA binding 2 (ID2).<sup>24-26</sup> In addition, activation of Notch1 inhibits PAX5 both at

transcriptional and post-translational levels.<sup>27</sup> Notch1 up-regulation is caused in part by stimulation by Jagged1, which is produced by the reactive cells surrounding the HRS cell.<sup>28,29</sup>

### **Survival and proliferation of HRS cells**

Nuclear factor kappa B (NF- $\kappa$ B) is a family of transcription factors which play a key role in numerous cellular responses including inflammatory responses and cell fate decisions.<sup>30</sup> This protein family includes five members, RelA (p65), RelB, c-Rel, NF- $\kappa$ B1 (p50 and its precursor p105) and NF- $\kappa$ B2 (p52 and its precursor p100), which function as homodimers and heterodimers.<sup>31</sup> In the absence of stimulation, NF- $\kappa$ B is maintained in an inactive state, mostly in the cytoplasm, by binding the inhibitory proteins I $\kappa$ B $\alpha$ , I $\kappa$ B $\beta$ , I $\kappa$ B $\epsilon$ , and the precursor proteins p105 and p100. NF- $\kappa$ B is activated following ligation of cellular receptors, including the tumour necrosis factor (TNF) receptor superfamily, resulting in I $\kappa$ B-kinase (IKK) activation and proteolytic degradation of I $\kappa$ Bs. The NF- $\kappa$ B complex is then free to bind its DNA target. Activation of NF- $\kappa$ B is normally transient and tightly controlled; however, in HRS cells NF- $\kappa$ B is constitutively active.<sup>32,33</sup>

NF- $\kappa$ B 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.<sup>34,35</sup> 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- $\kappa$ B activation in the HRS cell.<sup>36</sup> EBV can directly contribute to activation of NF- $\kappa$ B through its protein

LMP-1 which mimics CD40 signalling. Secondly, deleterious mutations of the genes encoding I $\kappa$ B proteins, in particular I $\kappa$ B $\alpha$ , have been described in 10-20% of cases of cHL.<sup>37-41</sup> Thirdly, amplification of the chromosomal region including the *c-Rel* gene has been reported in nearly 50% of cases of cHL cells.<sup>42-44</sup>

More recently, inactivating mutations of the *TNF $\alpha$ -induced protein 3* (*TNFAIP3*) gene, which encodes the protein A20, have been described in cHL.<sup>45,46</sup> A20 is a deubiquitinase and ubiquitinase which negatively regulates NF- $\kappa$ B and prevents excessive or prolonged activation.<sup>45</sup> Schmitz et al (2010)<sup>46</sup> 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- $\kappa$ B transcriptional activity and led to cytotoxicity. Kato and colleagues (2010)<sup>45</sup> 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- $\kappa$ B activation signal in HRS cells.<sup>46</sup>

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.<sup>47</sup> 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.,<sup>48-52</sup> Genomic lesions affecting the JAK-STAT pathway have also been demonstrated with copy number amplifications of the *JAK2* gene (9p24)<sup>53</sup> and mutations of *SOCS-1*, a negative regulator of JAK.<sup>54</sup> Several other signalling pathways have been shown to be deregulated in HL, including PI3K-Akt-mTOR,<sup>55</sup> MAPK-MEK-ERK<sup>56</sup> and the AP1-JUN/Fos pathway whose targets include CD30 and Galectin-1.<sup>57,58</sup>

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<sup>59</sup> 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.<sup>60</sup> Analysis of whole cHL tumours produced different results, probably reflecting alterations of microRNA profile in the infiltrating cells.<sup>61</sup> 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.<sup>53,62,63</sup> A recent array-based CGH analysis<sup>64</sup> 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.<sup>65</sup> 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.<sup>66,67</sup> 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.

### 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),<sup>68</sup> primary mediastinal B-cell lymphoma (PMBCL),<sup>69</sup> and “grey zone lymphoma”, which has features intermediate between DLBCL and cHL.<sup>70</sup> Gene expression profiling has identified PMBCL as being more closely related to cHL than DLBCL.<sup>71-73</sup> It has been shown that some of the key players involved in the pathogenesis of cHL, such as *REL*<sup>74,75</sup> and *JAK-STAT*<sup>76,77</sup>, 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 from 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.<sup>78</sup> Shafer and colleagues (2010)<sup>79</sup> 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.<sup>80</sup> 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.

**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

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<sup>81</sup>, CCL5 (RANTES) and CCL22.<sup>82</sup> 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<sup>83</sup> 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.<sup>84,85</sup> Secretion of galectin1<sup>58,86</sup> and chemokines including IL-10, TGF- $\beta$ ,<sup>83</sup> TARC, CCL5, CCL20 and CCL22.<sup>82,87</sup> by the HRS

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.<sup>88</sup>

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.<sup>89</sup> The eosinophils secrete TGF $\beta$ , and express CD30 ligand which stimulates CD30 expressed on the tumour cell surface<sup>90</sup>. Mast cells present in the infiltrate also express CD30 ligand, and in addition may contribute to angiogenesis.<sup>91</sup> 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.<sup>92,93</sup>

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<sup>62,94</sup>

Proteomic analysis of cell lines and primary tissue has also revealed elevated levels of TARC, IL1R2, MIF, CD26, CD44, and cathepsin S.<sup>95</sup> A summary of these, and other, molecules implicated in the cross-talk between HRS cells and their micro-environment is given in Table 1<sup>24,34,36,58,81,86,87,90,91,93,96,96-116</sup>

### **Epstein-Barr virus and Hodgkin lymphoma**

Epstein-Barr virus (EBV) is a lymphotropic  $\gamma$ -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 lead to the development of infectious mononucleosis (IM). Following primary infection,

the virus remains latent in memory B-cells for the lifetime of the host.<sup>117</sup> and is kept in check by the cytotoxic T-lymphocyte (CTL) response.<sup>118</sup> 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.<sup>4</sup> 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.<sup>119-121</sup> 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”<sup>117</sup> 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.<sup>122</sup> 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.<sup>98</sup> LMP-1 can mimic the signal provided by CD40, thus providing a means of constitutively activating NF- $\kappa$ B. LMP-1 is also capable of activating p38, PI3K, AP1 and JAK-STAT signalling.<sup>123</sup> 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.<sup>124-126</sup> LMP-1 and LMP-2 may also contribute to the reprogramming of the B-cell phenotype that occurs in HRS cells,<sup>127,128</sup> 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.<sup>129</sup> The BARTs are a group of alternatively spliced RNAs derived from the BamHI 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- $\kappa$ B, in particular *TNFAIP3* (A20), are more common in EBV-negative cases suggesting that these mutations substitute for LMP-1 expression<sup>46</sup>. 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<sup>130</sup> 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.,<sup>131,132</sup> Cases of mixed-cellularity subtype are also significantly more likely to be EBV-associated than nodular sclerosis cases.

There is an increased risk of EBV-associated HL following infectious mononucleosis,<sup>133,134</sup> 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,<sup>135</sup> 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.<sup>136</sup> 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.<sup>137</sup> 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.<sup>138,139</sup> Patients with EBV-associated HL were more likely to have reactivated VZV in the year prior to diagnosis than patients with EBV-negative disease.<sup>140</sup> 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.<sup>141</sup>

**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.<sup>142</sup> 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.<sup>118,143</sup> 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- $\kappa$ B pathway. EBV infection most likely leads to NF- $\kappa$ B activation in EBV-associated cases and mutations of genes encoding inhibitors and regulators of NF- $\kappa$ B 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.

**Acknowledgements**

The work of our laboratory is supported by Leukaemia and Lymphoma

Research. Katrina Farrell is funded by a Kay Kendall Leukaemia Fund Clinical

Research Fellowship. We would like to thank Dr. A. Gallagher for critical

reading of the manuscript.

For Peer Review

## Reference List

- (1) Information Services Division NHSS. Detailed incidence and mortality data, lifetime risk, prevalence and survival statistics: Hodgkins Disease. <http://www.isdscotland.org/isd/1473.html#summ> . 12-7-0010. 25-3-2010.
- (2) Hodgkin T. On some morbid appearances of the absorbent glands and spleen. *Med Chir Trans.* 1832;17:69-97.
- (3) Stein H, Poppema S, Delsol G et al. Hodgkin Lymphoma. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues.* Lyon: World Health Organisation; 2008:321-334.
- (4) Jarrett RF. Viruses and Hodgkin's lymphoma. *Ann Oncol.* 2002;13 Suppl 1:23-29.
- (5) Kuppers R, Rajewsky K, Zhao M et al. Hodgkin disease: Hodgkin and Reed-Sternberg cells picked from histological sections show clonal immunoglobulin gene rearrangements and appear to be derived from B cells at various stages of development. *Proc Natl Acad Sci U S A.* 1994;91:10962-10966.
- (6) Kuppers R, Hajadi M, Plank L, Rajewsky K, Hansmann ML. Molecular Ig gene analysis reveals that monocytoid B cell lymphoma is a malignancy of mature B cells carrying somatically mutated V region genes and suggests that rearrangement of the kappa-deleting element (resulting in deletion of the Ig kappa enhancers) abolishes somatic hypermutation in the human. *Eur J Immunol.* 1996;26:1794-1800.
- (7) Kanzler H, Kuppers R, Hansmann ML, Rajewsky K. Hodgkin and Reed-Sternberg cells in Hodgkin's disease represent the outgrowth of a dominant tumor clone derived from (crippled) germinal center B cells. *J Exp Med.* 1996;184:1495-1505.
- (8) Marafioti T, Hummel M, Foss HD et al. Hodgkin and reed-sternberg cells represent an expansion of a single clone originating from a germinal center B-cell with functional immunoglobulin gene rearrangements but defective immunoglobulin transcription. *Blood.* 2000;95:1443-1450.
- (9) Kuppers R. Molecular biology of Hodgkin's lymphoma. *Adv Cancer Res.* 2002;84:277-312.
- (10) Brauningner A, Schmitz R, Bechtel D et al. Molecular biology of Hodgkin's and Reed/Sternberg cells in Hodgkin's lymphoma. *Int J Cancer.* 2006;118:1853-1861.

- (11) Kuppers R, Sousa AB, Baur AS et al. Common germinal-center B-cell origin of the malignant cells in two composite lymphomas, involving classical Hodgkin's disease and either follicular lymphoma or B-CLL. *Mol Med*. 2001;7:285-292.
- (12) Muschen M, Rajewsky K, Brauninger A et al. Rare occurrence of classical Hodgkin's disease as a T cell lymphoma. *J Exp Med*. 2000;191:387-394.
- (13) Muschen M, Kuppers R, Spieker T et al. Molecular single-cell analysis of Hodgkin- and Reed-Sternberg cells harboring unmutated immunoglobulin variable region genes. *Lab Invest*. 2001;81:289-295.
- (14) Seitz V, Hummel M, Marafioti T et al. Detection of clonal T-cell receptor gamma-chain gene rearrangements in Reed-Sternberg cells of classic Hodgkin disease. *Blood*. 2000;95:3020-3024.
- (15) Hertel CB, Zhou XG, Hamilton-Dutoit SJ, Junker S. Loss of B cell identity correlates with loss of B cell-specific transcription factors in Hodgkin/Reed-Sternberg cells of classical Hodgkin lymphoma. *Oncogene*. 2002;21:4908-4920.
- (16) Schwering I, Brauninger A, Klein U et al. Loss of the B-lineage-specific gene expression program in Hodgkin and Reed-Sternberg cells of Hodgkin lymphoma. *Blood*. 2003;101:1505-1512.
- (17) Foss HD, Reusch R, Demel G et al. Frequent expression of the B-cell-specific activator protein in Reed-Sternberg cells of classical Hodgkin's disease provides further evidence for its B-cell origin. *Blood*. 1999;94:3108-3113.
- (18) Drexler HG. Recent results on the biology of Hodgkin and Reed-Sternberg cells. I. Biopsy material. *Leuk Lymphoma*. 1992;8:283-313.
- (19) Peh SC, Kim LH, Poppema S. TARC, a CC chemokine, is frequently expressed in classic Hodgkin's lymphoma but not in NLP Hodgkin's lymphoma, T-cell-rich B-cell lymphoma, and most cases of anaplastic large cell lymphoma. *Am J Surg Pathol*. 2001;25:925-929.
- (20) Ushmorov A, Leithauser F, Sakk O et al. Epigenetic processes play a major role in B-cell-specific gene silencing in classical Hodgkin lymphoma. *Blood*. 2006;107:2493-2500.
- (21) Ushmorov A, Ritz O, Hummel M et al. Epigenetic silencing of the immunoglobulin heavy-chain gene in classical Hodgkin lymphoma-derived cell lines contributes to the loss of immunoglobulin expression. *Blood*. 2004;104:3326-3334.
- (22) Nie L, Xu M, Vladimirova A, Sun XH. Notch-induced E2A ubiquitination and degradation are controlled by MAP kinase activities. *EMBO J*. 2003;22:5780-5792.

- (23) Smith EM, Akerblad P, Kadesch T, Axelson H, Sigvardsson M. Inhibition of EBF function by active Notch signaling reveals a novel regulatory pathway in early B-cell development. *Blood*. 2005;106:1995-2001.
- (24) Kuppers R, Klein U, Scherping I et al. Identification of Hodgkin and Reed-Sternberg cell-specific genes by gene expression profiling. *J Clin Invest*. 2003;111:529-537.
- (25) Renne C, Martin-Subero JI, Eickernjager M et al. Aberrant expression of ID2, a suppressor of B-cell-specific gene expression, in Hodgkin's lymphoma. *Am J Pathol*. 2006;169:655-664.
- (26) Mathas S, Janz M, Hummel F et al. Intrinsic inhibition of transcription factor E2A by HLH proteins ABF-1 and Id2 mediates reprogramming of neoplastic B cells in Hodgkin lymphoma. *Nat Immunol*. 2006;7:207-215.
- (27) Jundt F, Acikgoz O, Kwon SH et al. Aberrant expression of Notch1 interferes with the B-lymphoid phenotype of neoplastic B cells in classical Hodgkin lymphoma. *Leukemia*. 2008;22:1587-1594.
- (28) Ushmorov A, Leithauser F, Sakk O et al. Epigenetic processes play a major role in B-cell-specific gene silencing in classical Hodgkin lymphoma. *Blood*. 2006;107:2493-2500.
- (29) Ushmorov A, Ritz O, Hummel M et al. Epigenetic silencing of the immunoglobulin heavy-chain gene in classical Hodgkin lymphoma-derived cell lines contributes to the loss of immunoglobulin expression. *Blood*. 2004;104:3326-3334.
- (30) Ghosh S, May MJ, Kopp EB. NF-kappa B and Rel proteins: evolutionarily conserved mediators of immune responses. *Annu Rev Immunol*. 1998;16:225-260.
- (31) Jost PJ, Ruland J. Aberrant NF-kappaB signaling in lymphoma: mechanisms, consequences, and therapeutic implications. [Review] [96 refs]. *Blood*. 2007;109:2700-2707.
- (32) Bargou RC, Emmerich F, Krappmann D et al. Constitutive nuclear factor-kappaB-RelA activation is required for proliferation and survival of Hodgkin's disease tumor cells. *J Clin Invest*. 1997;100:2961-2969.
- (33) Bargou RC, Leng C, Krappmann D et al. High-level nuclear NF-kappa B and Oct-2 is a common feature of cultured Hodgkin/Reed-Sternberg cells. *Blood*. 1996;87:4340-4347.
- (34) Fiumara P, Snell V, Li Y et al. Functional expression of receptor activator of nuclear factor kappaB in Hodgkin disease cell lines. *Blood*. 2001;98:2784-2790.

- (35) Carbone A, Gloghini A, Gattei V et al. Expression of functional CD40 antigen on Reed-Sternberg cells and Hodgkin's disease cell lines. *Blood*. 1995;85:780-789.
- (36) Horie R, Watanabe T, Morishita Y et al. Ligand-independent signaling by overexpressed CD30 drives NF-kappaB activation in Hodgkin-Reed-Sternberg cells. *Oncogene*. 2002;21:2493-2503.
- (37) Emmerich F, Meiser M, Hummel M et al. Overexpression of I kappa B alpha without inhibition of NF-kappaB activity and mutations in the I kappa B alpha gene in Reed-Sternberg cells. *Blood*. 1999;94:3129-3134.
- (38) Emmerich F, Theurich S, Hummel M et al. Inactivating I kappa B epsilon mutations in Hodgkin/Reed-Sternberg cells. *J Pathol*. 2003;201:413-420.
- (39) Wood KM, Roff M, Hay RT. Defective IkappaBalpha in Hodgkin cell lines with constitutively active NF-kappaB. *Oncogene*. 1998;16:2131-2139.
- (40) Jungnickel B, Staratschek-Jox A, Brauninger A et al. Clonal deleterious mutations in the IkappaBalpha gene in the malignant cells in Hodgkin's lymphoma. *J Exp Med*. 2000;191:395-402.
- (41) Lake A, Shield LA, Cordano P et al. Mutations of NFKBIA, encoding IkappaB alpha, are a recurrent finding in classical Hodgkin lymphoma but are not a unifying feature of non-EBV-associated cases. *Int J Cancer*. 2009;125:1334-1342.
- (42) Joos S, Menz CK, Wrobel G et al. Classical Hodgkin lymphoma is characterized by recurrent copy number gains of the short arm of chromosome 2. *Blood*. 2002;99:1381-1387.
- (43) Barth TF, Martin-Subero JI, Joos S et al. Gains of 2p involving the REL locus correlate with nuclear c-Rel protein accumulation in neoplastic cells of classical Hodgkin lymphoma. *Blood*. 2003;101:3681-3686.
- (44) Martin-Subero JI, Gesk S, Harder L et al. Recurrent involvement of the REL and BCL11A loci in classical Hodgkin lymphoma. *Blood*. 2002;99:1474-1477.
- (45) Kato M, Sanada M, Kato I et al. Frequent inactivation of A20 in B-cell lymphomas. *Nature*. 2009;459:712-716.
- (46) Schmitz R, Hansmann ML, Bohle V et al. TNFAIP3 (A20) is a tumor suppressor gene in Hodgkin lymphoma and primary mediastinal B cell lymphoma. *J Exp Med*. 2009;206:981-989.
- (47) Murray PJ. The JAK-STAT signaling pathway: input and output integration. *J Immunol*. 2007;178:2623-2629.

- (48) Hinz M, Lemke P, Anagnostopoulos I et al. Nuclear factor kappaB-dependent gene expression profiling of Hodgkin's disease tumor cells, pathogenetic significance, and link to constitutive signal transducer and activator of transcription 5a activity. *J Exp Med.* 2002;196:605-617.
- (49) Skinnider BF, Elia AJ, Gascoyne RD et al. Signal transducer and activator of transcription 6 is frequently activated in Hodgkin and Reed-Sternberg cells of Hodgkin lymphoma. *Blood.* 2002;99:618-626.
- (50) Kube D, Holtick U, Vockerodt M et al. STAT3 is constitutively activated in Hodgkin cell lines. *Blood.* 2001;98:762-770.
- (51) Holtick U, Vockerodt M, Pinkert D et al. STAT3 is essential for Hodgkin lymphoma cell proliferation and is a target of tyrphostin AG17 which confers sensitization for apoptosis. *Leukemia.* 2005;936-944.
- (52) Trieu Y, Wen XY, Skinnider BF et al. Soluble interleukin-13Ralpha2 decoy receptor inhibits Hodgkin's lymphoma growth in vitro and in vivo. *Cancer Res.* 2004;64:3271-3275.
- (53) Joos S, Granzow M, Holtgreve-Grez H et al. Hodgkin's lymphoma cell lines are characterized by frequent aberrations on chromosomes 2p and 9p including REL and JAK2. *Int J Cancer.* 2003;103:489-495.
- (54) Weniger MA, Melzner I, Menz CK et al. Mutations of the tumor suppressor gene SOCS-1 in classical Hodgkin lymphoma are frequent and associated with nuclear phospho-STAT5 accumulation. *Oncogene.* 2006;25:2679-2684.
- (55) Dutton A, Reynolds GM, Dawson CW, Young LS, Murray PG. Constitutive activation of phosphatidylinositol 3 kinase contributes to the survival of Hodgkin's lymphoma cells through a mechanism involving Akt kinase and mTOR. *J Pathol.* 2005;205:498-506.
- (56) Zheng B, Fiumara P, Li YV et al. MEK/ERK pathway is aberrantly active in Hodgkin disease: a signaling pathway shared by CD30, CD40, and RANK that regulates cell proliferation and survival. *Blood.* 2003;102:1019-1027.
- (57) Mathas S, Hinz M, Anagnostopoulos I et al. Aberrantly expressed c-Jun and JunB are a hallmark of Hodgkin lymphoma cells, stimulate proliferation and synergize with NF-kappa B. *EMBO J.* 2002;21:4104-4113.
- (58) Juszczynski P, Ouyang J, Monti S et al. The AP1-dependent secretion of galectin-1 by Reed Sternberg cells fosters immune privilege in classical Hodgkin lymphoma. *Proc Natl Acad Sci U S A.* 2007;104:13134-13139.
- (59) Kluiver J, Poppema S, de Jong D et al. BIC and miR-155 are highly expressed in Hodgkin, primary mediastinal and diffuse large B cell lymphomas. *J Pathol.* 2005;207:243-249.

- (60) Van Vlierberghe P, De Weer A, Mestdagh P et al. Comparison of miRNA profiles of microdissected Hodgkin/Reed-Sternberg cells and Hodgkin cell lines versus CD77+ B-cells reveals a distinct subset of differentially expressed miRNAs. *Br J Haematol.* 2009;147:686-690.
- (61) Navarro A, Gaya A, Martinez A et al. MicroRNA expression profiling in classic Hodgkin lymphoma. *Blood.* 2008;111:2825-2832.
- (62) Kluiver J, Kok K, Pfeil I et al. Global correlation of genome and transcriptome changes in classical Hodgkin lymphoma. *Hematol Oncol.* 2007;25:21-29.
- (63) Chui DT, Hammond D, Baird M et al. Classical Hodgkin lymphoma is associated with frequent gains of 17q. *Genes Chromosomes Cancer.* 2003;38:126-136.
- (64) Steidl C, Telenius A, Shah SP et al. Genome-wide copy number analysis of Hodgkin Reed-Sternberg cells identifies recurrent imbalances with correlations to treatment outcome. *Blood.* 2010;e-publication ahead of print-2009.
- (65) Falzetti D, Crescenzi B, Matteuci C et al. Genomic instability and recurrent breakpoints are main cytogenetic findings in Hodgkin's disease. *Haematologica.* 1999;84:298-305.
- (66) Martin-Subero JI, Klapper W, Sotnikova A et al. Chromosomal breakpoints affecting immunoglobulin loci are recurrent in Hodgkin and Reed-Sternberg cells of classical Hodgkin lymphoma. *Cancer Res.* 2006;66:10332-10338.
- (67) Wlodarska I, Nooyen P, Maes B et al. Frequent occurrence of BCL6 rearrangements in nodular lymphocyte predominance Hodgkin lymphoma but not in classical Hodgkin lymphoma. *Blood.* 2003;101:706-710.
- (68) Stein H, Warnke RA, Chan WC et al. Diffuse large B-cell lymphoma, not otherwise specified. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues.* Lyon: World Health Organisation/ IARC; 2008:233-237.
- (69) Gaulard P, Harris NL, Pileri SA et al. Primary mediastinal (thymic) large B-cell lymphoma. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues.* Lyon: WHO; 2008:250-251.
- (70) Jaffe ES, Stein H, Swerdlow SH et al. B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H et al., eds. *WHO Classification of Tumours*

of Haematopoietic and Lymphoid Tissues. Lyon: World Health Organisation/ IARC; 2008:267-268.

- (71) Calvo KR, Traverse-Glehen A, Pittaluga S, Jaffe ES. Molecular profiling provides evidence of primary mediastinal large B-cell lymphoma as a distinct entity related to classic Hodgkin lymphoma: implications for mediastinal gray zone lymphomas as an intermediate form of B-cell lymphoma. *Adv Anat Pathol*. 2004;11:227-238.
- (72) Savage KJ, Monti S, Kutok JL et al. The molecular signature of mediastinal large B-cell lymphoma differs from that of other diffuse large B-cell lymphomas and shares features with classical Hodgkin lymphoma. *Blood*. 2003;102:3871-3879.
- (73) Rosenwald A, Wright G, Leroy K et al. Molecular diagnosis of primary mediastinal B cell lymphoma identifies a clinically favorable subgroup of diffuse large B cell lymphoma related to Hodgkin lymphoma. *J Exp Med*. 2003;198:851-862.
- (74) Poppema S, Kluiver JL, Atayar C et al. Report: workshop on mediastinal grey zone lymphoma. *Eur J Haematol Suppl*. 2005;45-52.
- (75) Weniger MA, Gesk S, Ehrlich S et al. Gains of REL in primary mediastinal B-cell lymphoma coincide with nuclear accumulation of REL protein. *Genes Chromosomes Cancer*. 2007;46:406-415.
- (76) Wessendorf S, Barth TF, Viardot A et al. Further delineation of chromosomal consensus regions in primary mediastinal B-cell lymphomas: an analysis of 37 tumor samples using high-resolution genomic profiling (array-CGH). *Leukemia*. 2007;21:2463-2469.
- (77) Ritz O, Guiter C, Castellano F et al. Recurrent mutations of the STAT6 DNA binding domain in primary mediastinal B-cell lymphoma. *Blood*. 2009;114:1236-1242.
- (78) Jones RJ, Gocke CD, Kasamon YL et al. Circulating clonotypic B cells in classic Hodgkin lymphoma. *Blood*. 2009;113:5920-5926.
- (79) Shafer JA, Cruz CR, Leen AM et al. Antigen-specific cytotoxic T lymphocytes can target chemoresistant side-population tumor cells in Hodgkin lymphoma. *Leuk Lymphoma*. 2010;51:870-880.
- (80) Schmitz R, Stanelle J, Hansmann ML, Kuppers R. Pathogenesis of classical and lymphocyte-predominant Hodgkin lymphoma. [Review] [150 refs]. *Annual Review Of Pathology*. 2009;4:151-174.
- (81) van den Berg AM, Visser L, Poppema S. High expression of the CC chemokine TARC in Reed-Sternberg cells. A possible explanation for the characteristic T-cell infiltrate in Hodgkin's lymphoma. *Am J Pathol*. 1999;154:1685-1691.

- (82) Skinnider BF, Mak TW. The role of cytokines in classical Hodgkin lymphoma. *Blood*. 2002;99:4283-4297.
- (83) Marshall NA, Christie LE, Munro LR et al. Immunosuppressive regulatory T cells are abundant in the reactive lymphocytes of Hodgkin lymphoma. *Blood*. 2004;103:1755-1762.
- (84) Li J, Qian CN, Zeng YX. Regulatory T cells and EBV associated malignancies. *Int Immunopharmacol*. 2009;9:590-592.
- (85) Marshall NA, Culligan DJ, Tighe J et al. The relationships between Epstein-Barr virus latent membrane protein 1 and regulatory T cells in Hodgkin's lymphoma. *Exp Hematol*. 2007;35:596-604.
- (86) Gandhi MK, Moll G, Smith C et al. Galectin-1 mediated suppression of Epstein-Barr virus specific T-cell immunity in classic Hodgkin lymphoma. *Blood*. 2007;110:1326-1329.
- (87) Aldinucci D, Lorenzon D, Cattaruzza L et al. Expression of CCR5 receptors on Reed-Sternberg cells and Hodgkin lymphoma cell lines: involvement of CCL5/Rantes in tumor cell growth and microenvironmental interactions. *Int J Cancer*. 2008;122:769-776.
- (88) Ma Y, Visser L, Blokzijl T et al. The CD4+CD26- T-cell population in classical Hodgkin's lymphoma displays a distinctive regulatory T-cell profile. *Lab Invest*. 2008;88:482-490.
- (89) Teruya-Feldstein J, Tosato G, Jaffe ES. The role of chemokines in Hodgkin's disease. *Leuk Lymphoma*. 2000;38:363-371.
- (90) Pinto A, Aldinucci D, Gloghini A et al. Human eosinophils express functional CD30 ligand and stimulate proliferation of a Hodgkin's disease cell line. *Blood*. 1996;88:3299-3305.
- (91) Aldinucci D, Gloghini A, Pinto A, De Filippi R, Carbone A. The classical Hodgkin's lymphoma microenvironment and its role in promoting tumour growth and immune escape. *J Pathol*. 2010;221:248-263.
- (92) Kapp U, Yeh WC, Patterson B et al. Interleukin 13 is secreted by and stimulates the growth of Hodgkin and Reed-Sternberg cells. *J Exp Med*. 1999;189:1939-1946.
- (93) Skinnider BF, Kapp U, Mak TW. The role of interleukin 13 in classical Hodgkin lymphoma. *Leuk Lymphoma*. 2002;43:1203-1210.
- (94) Gallagher A, Kluiver J, Andrew L et al. Serial Analysis of Gene Expression on primary Hodgkin and Reed-Sternberg Cells. *Haematologica*. 2007;92:41-42.
- (95) Ma Y, Visser L, Roelofsen H et al. Proteomics analysis of Hodgkin lymphoma: identification of new players involved in the cross-talk

- between HRS cells and infiltrating lymphocytes. *Blood*. 2008;111:2339-2346.
- (96) Teruya-Feldstein J, Jaffe ES, Burd PR et al. Differential chemokine expression in tissues involved by Hodgkin's disease: direct correlation of eotaxin expression and tissue eosinophilia. *Blood*. 1999;93:2463-2470.
- (97) Hedvat CV, Jaffe ES, Qin J et al. Macrophage-derived chemokine expression in classical Hodgkin's lymphoma: application of tissue microarrays. *Mod Pathol*. 2001;14:1270-1276.
- (98) Baumforth KR, Birgersdotter A, Reynolds GM et al. Expression of the Epstein-Barr virus-encoded Epstein-Barr virus nuclear antigen 1 in Hodgkin's lymphoma cells mediates Up-regulation of CCL20 and the migration of regulatory T cells. *Am J Pathol*. 2008;173:195-204.
- (99) Buri C, Korner M, Scharli P et al. CC chemokines and the receptors CCR3 and CCR5 are differentially expressed in the nonneoplastic leukocytic infiltrates of Hodgkin disease. *Blood*. 2001;97:1543-1548.
- (100) Atayar C, Poppema S, Blokzijl T et al. Expression of the T-cell transcription factors, GATA-3 and T-bet, in the neoplastic cells of Hodgkin lymphomas. *Am J Pathol*. 2005;166:127-134.
- (101) Ohshima K, Karube K, Hamasaki M et al. Imbalances of chemokines, chemokine receptors and cytokines in Hodgkin lymphoma: classical Hodgkin lymphoma vs. Hodgkin-like ATLL. *Int J Cancer*. 2003;106:706-712.
- (102) Kadin M, Butmarc J, Elovic A, Wong D. Eosinophils are the major source of transforming growth factor-beta 1 in nodular sclerosing Hodgkin's disease. *Am J Pathol*. 1993;142:11-16.
- (103) Newcom SR, Gu L. Transforming growth factor beta 1 messenger RNA in Reed-Sternberg cells in nodular sclerosing Hodgkin's disease. *J Clin Pathol*. 1995;48:160-163.
- (104) Yamamoto R, Nishikori M, Kitawaki T et al. PD-1-PD-1 ligand interaction contributes to immunosuppressive microenvironment of Hodgkin lymphoma. *Blood*. 2008;111:3220-3224.
- (105) Samoszuk M, Nansen L. Detection of interleukin-5 messenger RNA in Reed-Sternberg cells of Hodgkin's disease with eosinophilia. *Blood*. 1990;75:13-16.
- (106) Merz H, Houssiau FA, Orscheschek K et al. Interleukin-9 expression in human malignant lymphomas: unique association with Hodgkin's disease and large cell anaplastic lymphoma. *Blood*. 1991;78:1311-1317.

- (107) Jundt F, Anagnostopoulos I, Bommert K et al. Hodgkin/Reed-Sternberg cells induce fibroblasts to secrete eotaxin, a potent chemoattractant for T cells and eosinophils. *Blood*. 1999;94:2065-2071.
- (108) Hanamoto H, Nakayama T, Miyazato H et al. Expression of CCL28 by Reed-Sternberg cells defines a major subtype of classical Hodgkin's disease with frequent infiltration of eosinophils and/or plasma cells. *Am J Pathol*. 2004;164:997-1006.
- (109) Jucker M, Abts H, Li W et al. Expression of interleukin-6 and interleukin-6 receptor in Hodgkin's disease. *Blood*. 1991;77:2413-2418.
- (110) Foss HD, Herbst H, Gottstein S et al. Interleukin-8 in Hodgkin's disease. Preferential expression by reactive cells and association with neutrophil density. *Am J Pathol*. 1996;148:1229-1236.
- (111) Byrne PV, Heit WF, March CJ. Human granulocyte-macrophage colony-stimulating factor purified from a Hodgkin's tumor cell line. *Biochim Biophys Acta*. 1986;874:266-273.
- (112) Kretschmer C, Jones DB, Morrison K et al. Tumor necrosis factor alpha and lymphotoxin production in Hodgkin's disease. *Am J Pathol*. 1990;137:341-351.
- (113) Sappino AP, Seelentag W, Pelte MF, Alberto P, Vassalli P. Tumor necrosis factor/cachectin and lymphotoxin gene expression in lymph nodes from lymphoma patients. *Blood*. 1990;75:958-962.
- (114) Thorns C, Bernd HW, Hatton D et al. Matrix-metalloproteinases in Hodgkin lymphoma. *Anticancer Res*. 2003;23:1555-1558.
- (115) Pennanen H, Kuittinen O, Soini Y, Turpeenniemi-Hujanen T. Clinicopathological correlations of TIMP-1 and TIMP-2 in Hodgkin's lymphoma. *Eur J Haematol*. 2004;72:1-9.
- (116) O'Grady JT, Stewart S, Lowrey J, Howie SE, Krajewski AS. CD40 expression in Hodgkin's disease. *Am J Pathol*. 1994;144:21-26.
- (117) Rickinson A, Kieff E. Epstein-Barr Virus. In: Knipe DM, Howley PM, eds. *Field's Virology*, 5th Edition. Hagerstown, Maryland: Lippincott, Williams & Wilkins; 2007.
- (118) Hislop AD, Taylor GS, Sauce D, Rickinson AB. Cellular responses to viral infection in humans: lessons from Epstein-Barr virus. *Annu Rev Immunol*. 2007;25:587-617.
- (119) Gulley ML, Eagan PA, Quintanilla-Martinez L et al. Epstein-Barr virus DNA is abundant and monoclonal in the Reed-Sternberg cells of Hodgkin's disease: association with mixed cellularity subtype and Hispanic American ethnicity. *Blood*. 1994;83:1595-1602.

- (120) Weiss LM, Movahed LA, Warnke RA, Sklar J. Detection of Epstein-Barr viral genomes in Reed-Sternberg cells of Hodgkin's disease. *N Engl J Med.* 1989;320:502-506.
- (121) Weiss LM, Strickler JG, Warnke RA, Purtilo DT, Sklar J. Epstein-Barr viral DNA in tissues of Hodgkin's disease. *Am J Pathol.* 1987;129:86-91.
- (122) Jarrett RF. Viruses and lymphoma/leukaemia. *J Pathol.* 2006;208:176-186.
- (123) Young LS, Murray PG. Epstein-Barr virus and oncogenesis: from latent genes to tumours. *Oncogene.* 2003;22:5108-5121.
- (124) Bechtel D, Kurth J, Unkel C, Kuppers R. Transformation of BCR-deficient germinal-center B cells by EBV supports a major role of the virus in the pathogenesis of Hodgkin and posttransplantation lymphomas. *Blood.* 2005;106:4345-4350.
- (125) Chaganti S, Bell AI, Pastor NB et al. Epstein-Barr virus infection in vitro can rescue germinal center B cells with inactivated immunoglobulin genes. *Blood.* 2005;106:4249-4252.
- (126) Mancao C, Altmann M, Jungnickel B, Hammerschmidt W. Rescue of "crippled" germinal center B cells from apoptosis by Epstein-Barr virus. *Blood.* 2005;106:4339-4344.
- (127) Vockerodt M, Morgan SL, Kuo M et al. The Epstein-Barr virus oncoprotein, latent membrane protein-1, reprograms germinal centre B cells towards a Hodgkin's Reed-Sternberg-like phenotype. *J Pathol.* 2008;216:83-92.
- (128) Portis T, Dyck P, Longnecker R. Epstein-Barr Virus (EBV) LMP2A induces alterations in gene transcription similar to those observed in Reed-Sternberg cells of Hodgkin lymphoma. *Blood.* 2003;102:4166-4178.
- (129) Liu TY, Wu SJ, Huang MH et al. EBV-positive Hodgkin lymphoma is associated with suppression of p21cip1/waf1 and a worse prognosis. *Mol Cancer.* 2010;9:32.
- (130) Renne C, Willenbrock K, Martin-Subero JI et al. High expression of several tyrosine kinases and activation of the PI3K/AKT pathway in mediastinal large B cell lymphoma reveals further similarities to Hodgkin lymphoma. *Leukemia.* 2007;21:780-787.
- (131) Jarrett RF, Gallagher A, Jones DB et al. Detection of Epstein-Barr virus genomes in Hodgkin's disease: relation to age. *J Clin Pathol.* 1991;44:844-848.
- (132) Jarrett RF, Krajewski AS, Angus B et al. The Scotland and Newcastle epidemiological study of Hodgkin's disease: impact of histopathological

- review and EBV status on incidence estimates. *J Clin Pathol.* 2003;56:811-816.
- (133) Hjalgrim H, Smedby KE, Rostgaard K et al. Infectious mononucleosis, childhood social environment, and risk of Hodgkin lymphoma. *Cancer Res.* 2007;67:2382-2388.
- (134) Hjalgrim H, Askling J, Sorensen P et al. Risk of Hodgkin's disease and other cancers after infectious mononucleosis. *J Natl Cancer Inst.* 2000;92:1522-1528.
- (135) Khan G, Lake A, Shield L et al. Phenotype and frequency of Epstein-Barr virus-infected cells in pretreatment blood samples from patients with Hodgkin lymphoma. *Br J Haematol.* 2005;129:511-519.
- (136) Mueller N, Evans A, Harris NL et al. Hodgkin's disease and Epstein-Barr virus. Altered antibody pattern before diagnosis. *N Engl J Med.* 1989;320:689-695.
- (137) Landgren O, Gilbert ES, Rizzo JD et al. Risk factors for lymphoproliferative disorders after allogeneic hematopoietic cell transplantation. *Blood.* 2009;113:4992-5001.
- (138) Karunanayake CP, Singh GV, Spinelli JJ et al. Occupational exposures and Hodgkin Lymphoma: Canadian case-control study. *J Occup Environ Med.* 2009;51:1447-1454.
- (139) Tavani A, La Vecchia C, Franceschi S, Serraino D, Carbone A. Medical history and risk of Hodgkin's and non-Hodgkin's lymphomas. *Eur J Cancer Prev.* 2000;9:59-64.
- (140) Jarrett RF, Stark GL, White J et al. Impact of tumor Epstein-Barr virus status on presenting features and outcome in age-defined subgroups of patients with classic Hodgkin lymphoma: a population-based study. *Blood.* 2005;106:2444-2451.
- (141) Nakamura S, Jaffe ES, Swerdlow SH. EBV positive diffuse large B-cell lymphoma of the elderly. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues.* Lyon: WHO; 2008:243-244.
- (142) Hjalgrim H, Rostgaard K, Johnson PC et al. HLA-A alleles and infectious mononucleosis suggest a critical role for cytotoxic T-cell response in EBV-related Hodgkin lymphoma. *Proc Natl Acad Sci U S A.* 2010;107:6400-6405.
- (143) Straathof KC, Leen AM, Buza EL et al. Characterization of latent membrane protein 2 specificity in CTL lines from patients with EBV-positive nasopharyngeal carcinoma and lymphoma. *J Immunol.* 2005;175:4137-4147.

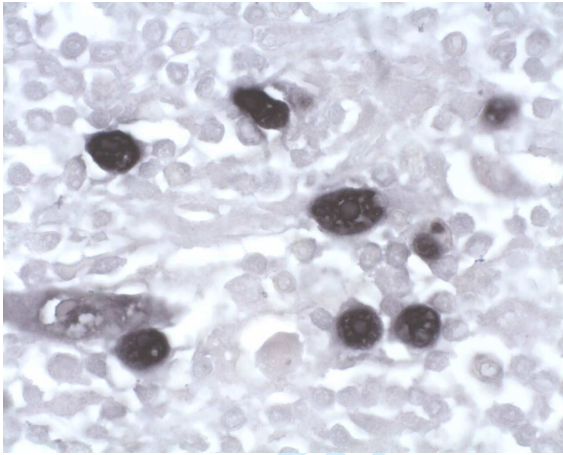
- (144) Mani H, Jaffe ES. Hodgkin lymphoma: an update on its biology with new insights into classification. [Review] [204 refs]. *Clinical Lymphoma & Myeloma*. 2009;9:206-216.
- (145) Rouillet MR, Bagg A. Recent insights into the biology of Hodgkin lymphoma: unraveling the mysteries of the Reed-Sternberg cell. [Review] [164 refs]. *Expert Review of Molecular Diagnostics*. 2007;7:805-820.

For Peer Review

Table 1: Cytokines, chemokines, receptors and ligands altered in classical Hodgkin lymphoma<sup>144,145</sup>

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- $\beta$	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- $\beta$	102,103
	TNF- $\alpha$	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- $\kappa$ B	CD40/CD40-R	116
	CD30/CD30-R	36,90
	TNF- $\alpha$ -	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



For Peer Review

Table 1: Cytokines, chemokines, receptors and ligands altered in classical Hodgkin lymphoma<sup>144,145</sup>

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- $\beta$	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- $\beta$	102,103
	TNF- $\alpha$	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- $\kappa$ B	CD40/CD40-R	116
	CD30/CD30-R	36,90
	TNF- $\alpha$ -	112,113
	RANK/RANK-L	34

For Peer Review

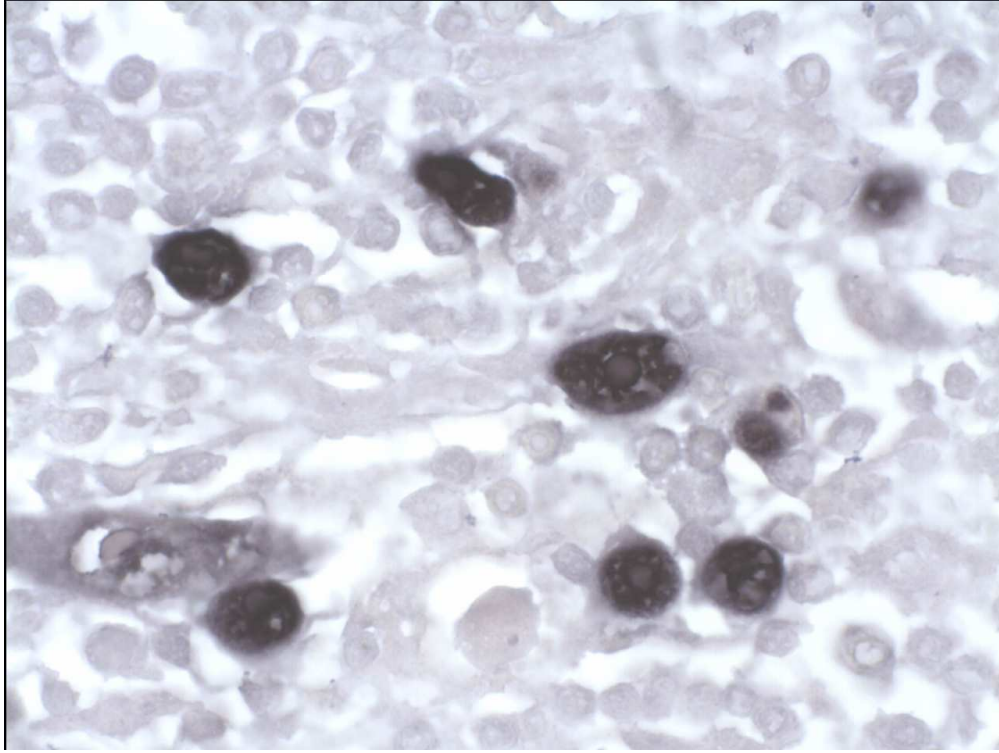


Figure 1: EBV EBER in situ hybridisation showing positive staining in the nuclei of all the tumour cells. x1000  
216x162mm (150 x 150 DPI)

review