Hodgkin Disease and the Role of the Immune System

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Abstract

Hodgkin disease (HD) is a malignancy of primarily B lymphocytes, which has the unique ability to cause immunodeficiency, as well as provide immune evasion mechanisms to avoid self-destruction. In this review, we will discuss Hodgkin disease, its association with EBV, the immune deficiency caused by HD, tumor immune evasion mechanisms. Specifically, we will closely evaluate the roles of regulatory T cells in HD, cytotoxic T cells, cytokine and chemokine secretion, downregulation of Fas ligand and IDO secretion.

Keywords

Hodgkin disease; regulatory T cells; EBV; cytotoxic T cells; chemokines; IDO secretion

Hodgkin Disease Background

Hodgkin disease (HD) is a malignant neoplasm of lymphoreticular cell origin, characterized by the presence of large mononucleated Hodgkin (H) and giant multinucleated Reed-Sternberg (RS) cells, collectively referred to as Hodgkin and Reed-Sternberg cells (HRS).12 Approximately 7,500 new cases are diagnosed in the United States annually, with the highest incidence in the 3rd decade of life.3 In the United States alone, the annual incidence is 7 cases per million children younger than 15 years of age.4 Hodgkin disease has a unique bimodal age distribution that differs both geographically and ethnically. In developing countries, the early peak occurs before adolescence, whereas in industrialized countries, the early peak occurs in the mid to late 20s with the second peak after the age of 50 years.5 Overall, there is a slight male predominance.6

Hodgkin disease is unique in that its malignant cells account for less than 1% of the total cell population of the tumor. The majority of tumor cells are composed of reactive inflammatory cells including lymphocytes, histiocytes, eosinophils, neutrophils, plasma cells and fibroblasts, which develop as a result of cytokine release. Found within these non-malignant reactive cells are the malignant HRS cells. In a pivotal paper by Kuppers et al in 1994, HRS cells were proven to be clonally related B-cell derived malignant cells.7

The classification system of HD has however changed in recent years. In 1994, the Rye system was incorporated into the Revised European–American Lymphoma (REAL) classification system, which separates HD into classical Hodgkin lymphoma (cHL) or nodular lymphocyte-predominant HL (LPHL) depending on the detection of HRS cells, or lymphocytic and histiocytic (L&H) cells, respectively. The cHL subtype includes NS, MC and LD and comprises 95% of cases, whereas the LPHL includes only the LP variant

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representing 5% of cases.\(^8\) Both subtypes derive from germinal center B cells in most instances, while in a few cases, cHL is T-cell derived.\(^9\) As these two different classifications of Hodgkin disease are actually quite distinct from one another and are associated with differing biologies and prognoses, we will focus this chapter only on the classical HL, which is more common and is associated with the characteristic HRS cells.

### The Role of EBV and the Immune System in Hodgkin Disease

Epstein-Barr virus (EBV) is the only infectious agent that consistently has been associated with HD, and notably EBV-encoded RNA is detected in the HRS cells in up to 40% of cases.\(^10–12\) A large proportion of patients with HD have high EBV antibody titers, suggesting that EBV infection may precede the development of HD, and clonality studies indicate EBV infection precedes the expansion of the tumor cell population.\(^13\) The incidence of EBV-associated HD varies by age, sex, ethnicity, histological subtype and economic level. It is present in 93% of Asian, 86% of Hispanic, 46% of Caucasian and 17% of African-American children with HD.\(^14;15\) Histologic subtypes also differ in their EBV-association, and the MC subtype is most commonly associated with EBV. In the United States and Europe, the MC subtype accounts for 30% of HD and most commonly occurs in males. In third world and developing countries, HD occurs more commonly in childhood, and the MC histology accounts for a larger percentage of cases as indicated by several reports from Central and South America.\(^16–18\)

Given that the peak incidence of infectious mononucleosis in the United States and Europe is in adolescents and young adults, it is therefore surprising that patients presenting in this age group are more likely to have EBV-negative disease. There does however, appear to be evidence that relatively recent infection with EBV is specific to EBV-positive HD,\(^19\) which raises the possibility that genetic susceptibility within the host has resulted in a defective immune response allowing proliferation and malignant transformation of EBV-infected cells.

### The Role of Nuclear Factor-Kappa B and LMP1

The EBV gene expression pattern of EBNA-1, BARFO, LMP1 and LMP2 (type 2 latency) is the hallmark of EBV-positive HD. Latent Membrane Protein 1 (LMP1) is a EBV viral protein and a member of the Tumor Necrosis Factor Receptor (TNFR) superfamily, which activates the transcription factor Nuclear Factor-KappaB (NF-kappaB) and modulates apoptotic and growth pathways.\(^20\) NF-kappaB activation and subsequent transcription results in regulating many activities including the immune system, cell proliferation, tumor metastasis, inflammation and viral replication.\(^21\) Numerous studies have shown that malignant HRS cells aberrantly express the activated p50/p65 (RelA) heterodimer form of NF-kappaB, which is critical for cell survival.\(^22–24\)

### EBV-positive HD and Genetic Predisposition

Considerable evidence exists supporting a chronic EBV process in HD;\(^26\) however, this strong connection is seen much less frequently in tumors from familial HD cases. Specifically, EBV-encoded RNA has been detected in only 27% of tumors from familial HD patients.\(^27\) Interestingly, this association is weakest with the nodular sclerosis subtype, which is the subtype most often represented among adolescents and young adults as well as familial cases.\(^28;29\) However, as >80% of healthy individuals are EBV positive and the incidence of HD is only 3 per 100,000 in developed countries, there is a suggestion that some genetic predisposition within the host has allowed these infected cells to proliferate unchecked by the immune response. Some studies have suggested that HLA Class I polymorphisms are susceptibility markers for EBV-positive HD, which in turn affects the
way in which the immune system deals with EBV-infected cells. Studies have also shown that part of the HLA class I and HLA class II regions are associated with susceptibility to EBV-positive HD. Therefore, these associations with the HLA system raise the possibilities that (a) the patient’s HLA type could affect the affinity of viral peptide binding, and hence the affinity of the T cells capable of recognizing the EBV antigen (e.g. LMP1 and/or LMP2) or that (b) regulatory T cells (Tregs) are selectively recruited by certain EBV peptides preferentially expressed by particular HLA-restricted antigens. Whatever the explanation, there does appear to be a relationship between HLA genotype and the risk of EBV-positive HD, and that the immune status of the patient critically influences the likelihood of suffering from EBV-positive HD.

Immune Status in Hodgkin Disease

T- and B-Cell Dysfunction

Patients with HD have a well-described cellular immune deficiency, which is based on clinical and laboratory findings. The underlying immune deficiency may be congenital or acquired with an increased incidence of HD in patients with combined variable immune deficiencies and HIV infection or may be related to the disease itself. In patients without obvious congenital or acquired causes, the onset of immune deficiency precedes the diagnosis of HD, worsens with advanced disease and improves when remission is induced. It is unclear whether the underlying immune deficiency is a primary or secondary phenomena in these patients, but the abnormality is certainly present early and even is shown to persist in some long-term survivors of HD. Clinically, the immune deficiency was originally defined by increased susceptibility to infections (bacterial, fungal and viral) in patients even before the initiation of cytotoxic chemotherapy and radiotherapy and although untreated HD patients appear to have adequate absolute numbers of B cells, there appears to be an intrinsic functional B lymphocyte defect in these patients.

Immune Deficiency and Genetic Predisposition

One frequently asked question is whether there is a genetic etiology to this apparent disease-associated immune deficiency. It is estimated that up to 4.5% of HD cases are familial. An elevated risk of HD among monozygotic twins compared with dizygotic twins has been noted in HD patients, suggesting a role for shared genetic factors in familial HD. Some studies have shown impaired T-cell responses in healthy twins and family members, yet others have not confirmed this finding. As discussed previously, the HLA region has been well implicated in HD, and the HLA class I region in chromosome 6 (especially A1, B5, B8, and B18 alleles) consistently has been associated with both sporadic and familial Hodgkin disease. Certainly, this link between the impaired immune response in the tissues and the generalized immune deficiency in HD patients is most likely multifactorial. However, it is also well established that the HRS cell employs numerous strategies by which to evade the immune response. Therefore, it may be that this overwhelming number of immune suppressive mechanisms leads to a general impairment in immune status in these patients. (Figure 1)

Tumor Immune Evasion Strategies in Hodgkin Disease

Restricted Pattern of Antigens

EBV-positive HD is a good example where the patient’s immune system has failed to eradicate the HD tumor cells, despite the expression of viral antigens by the tumor cells. One reason for this is the restricted array of EBV antigens expressed by the tumor cells. The viral gene expression on HRS cells is limited to the immunosubdominant latent membrane proteins (LMP) 1 and 2, BARFO, EBNA 1, which possess gly-ala repeat sequences that
inhibit HLA class I antigen processing, and small non-polyadenylated RNAs termed EBV early RNA (EBER) 1 and 2, which are transcribed but not translated.\textsuperscript{47–49} It is suggested that LMP1 and LMP2 peptides are not competitive with other EBV latent cycle peptides for binding to a patient’s HLA antigens, thus resulting in a poor immunogenic stimulus to LMP-specific CTLs. This expression of a minimal subset of genes, which are weak targets for CTL activity, therefore allows the malignant cells to evade the immune system.\textsuperscript{50}

**Regulatory T Cells**

In recent years, the role of regulatory T cells (Tregs) has stimulated a lot of interest as a possible cause of this immune suppression. Tregs play a role in the control of autoimmunity and transplantation rejection, but may also inhibit effective anti-tumor immune responses.\textsuperscript{51,52}

**The Role of CD4\(^+\) CD25\(^+\) Regulatory T Cells in Hodgkin Disease**

To test the hypothesis that regulatory T cell activity is important in HD, Marshall et al characterized the CD4\(^+\) T-cell responses and phenotypes in Hodgkin lymphoma-infiltrating lymphocytes (HLILs) and postulated that such activity could mediate the immunosuppression associated with HD and contribute to immune evasion by HRS cells.\textsuperscript{53} Their results found that HLILs are hyporesponsive to mitogens and antigens and suppress PBMC activation. They noted that the number of cells with a CD4\(^+\) CD25\(^+\) suppressor phenotype is more numerous in HLILs compared to healthy control donor PBMCs. In addition, they reported that the HD nodal lymphocytes are anergic and can also profoundly inhibit Th cell responses. Thus, their results indicated that regulatory T cells are the dominant T-cell population in HLILs.

Since recent studies have suggested the presence of Tregs in the reactive infiltrate may explain the inhibition of the antitumoral host immune response in HD patients, others have attempted to assess the relevance of these Tregs and CTLs. Álvaro et al investigated the possible association between the presence of Tregs in the infiltrate of cHL tumors, the presence of other immune cells and the effect of the presence of regulatory and cytotoxic T cells on the survival of HD patients.\textsuperscript{54} A Kaplan-Meier analysis of survival of these patients, taking into account the cell composition of the reactive background of lymphocytes, indicated that a high number of Foxp3\(^+\) cells was a significant predictor of longer event-free survival (RR, 2.296; \(P<0.05\)) and disease-free survival (RR, 2.852; \(P<0.05\)). However, cHL samples with a larger proportion of TIA-1\(^+\) cells in the tumor infiltrate was associated with a more aggressive clinical course, with a lower overall survival (RR, 4.644; \(P<0.01\)), lower EFS (RR, 2.582; \(P<0.01\)) and DFS (RR, 2.346; \(P<0.05\)). They also concluded that the presence of low Foxp3\(^+\) combined with the presence of high TIA-1\(^+\) cells correlated with an independent prognostic factor that negatively influenced event-free and disease-free survival.

**The Role of PD-L1 and PD-1-Mediated Immune Suppression in Hodgkin Disease**

**PD-1**—A recently identified mechanism of immune evasion is the inhibitory molecule programmed death-1 (PD-1) and its ligand PD-L. PD-1, is a member of the CD28 costimulatory receptor superfamly. T cells expressing the inhibitory molecule PD-1 show characteristics of “exhaustion:” anergy and limited cytokine secretion. Moreover, PD-1 has since been established as a marker for T-cell dysfunction and has been demonstrated as a poor prognostic factor in many diseases, including HD.\textsuperscript{55,56}

**PD-L**—PD-L (consisting of both B7-H1 and B7-DC) acts as the ligand for PD-1. Engagement of PD-L with PD-1 mitigates the cytolytic function, cytokine secretion, and proliferation of T cells.\textsuperscript{57,58} Studies using immunohistochemistry have demonstrated that
both EBV+ and EBV-HRS cells express PD-Ls and strongly suggest that the “exhaustion” of TILs is involved in the pathogenesis of HD and contributes to the highly immunosuppressive environment.

Role of Cytokine Secretion

Transforming Growth Factor-Beta

Most tumors secrete cytokines in an attempt to evade the host’s immune response. The majority of reactive cells involved in HD are T lymphocytes, and the cytokines produced by these non-malignant cells can support tumor proliferation and survival.59 The most potent and most widely employed immunosuppressive cytokine with direct anti-proliferative and anti-cytotoxic effects on cytotoxic T cells is TGF-β.60 Hence, secretion of this cytokine by malignant cells such as HRS tumor cells and tumor-infiltrating regulatory T cells may diminish the effectiveness of anti-tumor T-cell immune responses.

Interleukins

Several cytokines have been implicated in the promotion of growth in HD. Previous reports have noted that several cytokines are expressed by HD cell lines and by HRS cells in biopsy material, including interleukin IL-1, IL-5, IL-6, IL-7, IL-9, IL-10, granulocyte and monocyte colony stimulating factor (GM-CSF), lymphotoxin-α, and TGF-β.61 Many of the clinicopathologic features of HD, such as fevers, night sweats and eosinophilia, can be explained by this imbalance of cytokine production.62 Furthermore, IL-10 is able to inhibit T-cell proliferation, IFN-γ and IL-2 production by Th2–like T cells.63 Human IL-10 can be found in EBV-positive HRS cells64 and clinical studies in pre-treated HD patients have found that elevated sera levels of IL-10 predict an adverse and unfavorable response to treatment.65;66

Interleukin-13

IL-13 is a Th2 cytokine with homology to IL-4. Together, these cytokines play an important role in the coordination of the humoral immune response via their activities on B cells.67 IL-13 has immunomodulatory and anti-inflammatory effects mediated through its direct effects on B cells and monocyte/macrophage cells.68–72 IL-13 has been shown to be an autocrine growth factor in HD and is responsible for STAT6 activation, which is involved in the proliferation of normal B and T lymphocytes.73

Role of Chemokine Secretion

Chemokines are cytokines with chemoattractant properties. The chemokines produced by HRS cells play an integral role in leukocyte trafficking. These molecules contribute to the maintenance of a favorable survival environment in HRS cells. It has been postulated that the reactive infiltrate, which makes up the bulk of the tumor burden in HD, is due to the production of cytokines and chemokines produced by the malignant HRS cells.74–76 Chemokine studies have reported the presence of IL-8 (CXCL8) in stimulated HD cell lines and sera of HD patients.77;78 Interferon-inducible protein-10 (IP-10) is a chemoattractant for activated T-cells expressing the CXCR3 chemokine receptor.79 Previous studies of HD have reported an association between IP-10 expression in HRS cells and the mixed cellularity variety of HD.80–82 Additionally, these studies also have reported stronger expression of IP-10 in EBV-positive HD than in EBV-negative HD.83 Thus, it appears likely that LMP1 expression in HRS cells may contribute to the expression of IP-10 in these cells.

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Downregulation of Fas-ligand

Fas ligand (FasL) is a type II transmembrane protein member of the TNF superfamily and is expressed by activated T lymphocytes and natural killer (NK) cells as well as by a small number of non-lymphoid cells. By engaging its receptor (Fas), membrane-bound FasL induces apoptosis in the target cell, thereby playing a central role in both cell-mediated immunity and immune downregulation. The Fas/FasL pathway is one of the most crucial mechanisms for the induction of apoptosis in memory-effector T cells after the elimination of antigens. However, since the discovery that several tumors, including HD, can express FasL, the activation of this pathway has been considered a mechanism by which tumors expressing FasL escape destruction by the immune response.

Secretion of Indoleamine 2, 3-dioxygenase

Another mechanism of tumor immune evasion is via the secretion of prostaglandin E2 (PGE2) and indoleamine 2,3-dioxygenase (IDO). The latter is the enzyme responsible for the initial and rate-limiting step involved in the conversion of the essential amino acid tryptophan to N-formylkynurenine. Monocytes from HD patients are dysfunctional in their generation of oxygen radicals as well as candidacidal activity. This dysfunction has been shown to be associated with excessive production of PGE-2 by these cells. This increased secretion of PGE2 by monocytes in HD patients in turn upregulates IDO, which is also secreted by monocytes, dendritic cells and tumor cells. IDO activation limits the availability of tryptophan, and because tryptophan is required for protein synthesis, withdrawal of this essential amino acid from the micro-environment arrests protein biosynthesis and subsequent growth of pathogens and proliferating cells. Low levels of tryptophan at the tumor site causes T cells to arrest in the G1 phase of the cell cycle. In patients with tumors, it has been suggested that tryptophan degradation may represent an intrinsic immune escape mechanism of tumor cells. It has been proposed that enhanced endogenous formation of IFN-γ during the host’s anti tumor immune response leads to activation of IDO in monocyte-derived macrophages and/or dendritic cells, which in turn suppresses T-cell proliferation and act as an immunosuppressant.

Conclusion

It is well known that the immune system defense mechanisms are the least effective and final barrier in our natural defenses against carcinogenesis. The “immune surveillance theory” postulated by Sir Macfarlane Burnet and Lewis Thomas in the 1970s implied that immune response to tumors occurred very early in tumor stage development. Many studies have evolved in support of this theory and evidence now exists to suggest that when some tumors do grow, they are believed to have escaped from immune surveillance. It is also now well known that Hodgkin disease has numerous ways of avoiding destruction by immune evasive mechanisms and most likely this immune derangement is multifactorial. However, the fact that the Hodgkin tumor relies on so many mechanisms to evade the immune response raises the possibility that overcoming at least some of them may allow improved anti-tumor immunity in these patients. Therefore, a better understanding of such mechanisms will undoubtedly lead to more potential novel strategies to overcome this dilemma in patients with Hodgkin disease.

Reference List


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Figure 1. Tumor Immune Escape Mechanisms in Hodgkin Disease
Secretion of cytokines and chemokines by the tumor cells plays a strong role in the tumor’s ability to escape the immune system. For example, secretion of TGF-β inhibits maturation of CTLs, as do regulatory T cells, while IL-13 directly promotes the growth and survival of HD cell lines. TARC and IL-10 negatively affect CTL and APC activity, leading to unregulated growth of HD tumor cells. CD30 shed by HRS cells interferes between lymphocytes, cytokines and APCs. Activation of FasL pathway by HD tumor cells is a mechanism by which tumors expressing FasL escape destruction by the immune system. Secretion of PGE2 by monocytes in HD patients upregulates IDO, leading to tryptophan depletion, which is regarded as a defense mechanism induced by IFN-γ, thereby suppressing T-cell proliferation.