Review Article

Immune Escape Mechanisms in Diffuse Large B-Cell Lymphoma

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Diffuse large B-cell lymphoma (DLBCL) is the most frequent subtype of non-Hodgkin lymphomas in Western countries. Implementation of immunotherapy using monoclonal antibodies to therapeutic protocols has led to dramatic improvements in overall survival. DLBCL became a model of a successful immunochemotherapy concept. Despite this fact, there is still a proportion of patients who do not respond to or relapse early after treatment. Growing evidence suggests that host antitumor immunity is suppressed by lymphoma cells in many ways. First, host cytotoxic T cells are directly suppressed by interaction with programmed cell death (PD) ligand on lymphoma cell surface and a similar mechanism enhances the activity of suppressive regulatory T cells (Tregs). Second, tumor cells escape host cytotoxic cells due to lower immunogenicity caused by reduced expression of HLA antigens. Both mechanisms have an origin in primary genetic events in lymphomagenesis. Rearrangement of MHC class II transcriptional activator (CIITA) gene and amplification of Janus kinase (JAK2) gene lead to enhanced expression of PD ligands 1 and 2, higher proliferation activity, and lower expression of HLA. This paper summarizes current knowledge about clinically relevant immune escape mechanisms in DLBCL.

1. Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most frequent subtype of aggressive non-Hodgkin lymphomas (NHL) in the Western countries [1]. Implementation of immunotherapy using monoclonal antibodies to therapeutic protocols led to dramatic improvements in overall survival. DLBCL became a model of a successful immunochemotherapy concept [2, 3]. Despite this fact, there is still a proportion of patients who do not respond to or relapse early after treatment. Growing evidence suggests that host antitumor immunity is suppressed by lymphoma cells in many ways [4]. In many cases of resistant lymphoma patients, suppression of host immunity is assumed to play a critical role.

Antitumor immunity is one of the basic tasks for both the naive and adaptive immune systems. Suppression of antilymphoma effector cells, mainly T cells, is one of the key prerequisites for tumor invasion and growth [5]. Many years ago, lymphopenia was recognized as an independent negative predictor of survival in Hodgkin lymphoma [6]. The last years have brought numerous studies, with similar results obtained in DLBCL patients treated in the immunotherapy era [7, 8].

Recent preclinical studies show that there is a close relationship between tumor and host immunity. Host cytotoxic CD8+ T cells are directly suppressed by interaction with programmed cell death (PD) ligand on lymphoma cell surface and a similar mechanism enhances the activity of suppressive CD4+ regulatory T cells (Tregs) [9]. On the other hand, chemotherapy rescues tumor-driven aberrant CD4+ T-cell differentiation and restores an activated polyfunctional helper phenotype [10]. Another mechanism to effectively escape host cytotoxic cells is immunogenicity lowering due to reduced expression of human leukocyte antigens (HLA) [11]. These mechanisms originate in primary genetic events in lymphomagenesis. Rearrangement of the major histocompatibility complex (MHC) class II transcriptional activator (CIITA) gene and amplification of Janus kinase 2 (JAK2) gene have recently been recognized as promoters of both host immune suppression and immune escape [12].
Altogether, our knowledge about tumor microenvironment is becoming increasingly comprehensive. Many aspects of the relationship between tumor and host immunity are better recognized and intensively studied using molecular cytogenetic techniques, tissue microarrays, and immunohistochemistry methods.

This paper synthesizes current knowledge about clinically relevant immune escape mechanisms in DLBCL from the perspectives of cytogeneticists, immunopathologists, and hematopathologists.

2. Genetic Alterations and Immune Mechanisms in DLBCL

Important insights into the molecular pathogenesis of DLBCL have been achieved with the introduction of microarrays technology. Gene expression profiling studies revealed three molecular subtypes, termed germinal center B-cell-like (GCB) DLBCL, activated B-cell-like (ABC) DLBCL, and primary mediastinal B-cell lymphoma (PMBCL), which are indistinguishable using conventional diagnostic tools [13]. The Leukemia and Lymphoma Molecular Profiling Project (LLMPP) reported further results from multi-institutional gene expression microarray studies. This consortium described 4 gene expression signatures associated with outcome in DLBCL including the proliferation, MHC class II, lymph node (host response), and germinal center signatures [14].

MHC II genes are a family of genes mostly expressed from a single locus on chromosome 6p12. These genes are constitutively expressed in antigen-presenting cells, such as B cells, dendritic cells, monocytes, and macrophages, with inducible expression in many other cell types. The expression analysis in DLBCL cases also demonstrated that decreasing MHC class II expression was highly predictive of worse patient survival [14]. However, the mechanisms of lost MHC class II expression in most cases of DLBCL are unknown. One of the mentioned mechanisms in a particular subset of DLBCL is large hemizygous or homozygous deletions involving the MHC class II locus, but such a mechanism has not been confirmed [12]. More recently, small deletion or point mutation of the CIITA transcriptional regulator was published [15]. This observation leads to consideration that a more likely explanation of decreasing expression of MHC class II is decreased transcription as directed by the master transactivator molecule CIITA. The MHC class II genes all have similar sites in their regulatory regions for the cooperative binding of a transcriptosome complex of RFX, NF-Y, and CREB. This complex in turn creates a protein-binding site for CIITA, which does not bind DNA directly [15, 16]. CIITA (encoded by the MHC2TA gene) expression is usually highly correlated with MHC class II expression. It was confirmed that loss of functional expression of any of the 3 genes encoding the subunits of the heterotrimer RFX or of the MHC2TA gene encoding CIITA leads to elimination of all MHC class II expressions, as evidenced in the congenital immunodeficiency diseases known as the MHC II deficiency syndromes [17].

Disruption of CIITA was shown to downregulate surface levels of MHC class II, a phenomenon known to reduce tumor immunogenicity. A recent study using molecular genetic and cytogenetic methods revealed that rearrangement of CIITA is present in up to 3% of DLBCL but up to 38% of PMBCL and that the translocation plays a key role in the molecular pathogenesis of B-cell lymphomas [18].

The CIITA gene is a highly promiscuous gene involved in rearrangements and a number of genes acting as the fusion partners of translocation were detected. One such translocation partner is gene BX628577 localized on chromosome 15 (15q21.3). Steidl and Gascoyne [19] studied this translocation in the KM-H2 cell line. They confirmed high expression of fusion transcripts and obtained data demonstrating that the CIITA-BX628577 fusion suppresses expression of HLA class II genes in the KM-H2 cell line. They also analyzed a cohort of 54 PMBCL patients and discovered five new fusion partners and breakpoints in CIITA. They recognized that these changes are novel and highly recurrent in PMBCL. The study also confirmed that the presence of a breakpoint in CIITA conferred a statistically significant survival disadvantage, presumably because of reduced immunemediated control of the tumor.

A subsequent functional study suggests that escape from immunosurveillance through various mechanisms might play an important role in the pathogenesis of lymphomas. In PMBCL cases, the 9p24 multiplication involving the JAK2 gene is a frequent and nonrandom aberration detected in up to 63% of cases. It was confirmed that amplification leads to overexpression of the gene to constitutive activation of JAK2 kinase and thus to uncontrolled cell proliferation. In their recent study, Green et al. [20] confirmed that JAK2 amplification is accompanied by amplification of immunoregulatory genes PD ligands 1 and 2 in the PMBCL cell line as both genes are located in the amplification region together with the JAK2 gene. Further analysis of primary tumors confirmed association between amplification of 9p24.1 and increased expression of PD-1 ligands, particularly PD-L2 in PMBCL. Products of these PDL1/PDL2 genes are immunoregulatory proteins belonging to the same family of receptors that affect T-cell activity. These results also confirm that 9p24.1 amplification is a disease-specific genetic abnormality of prognostic and therapeutic importance. Involvement of different immunoregulatory cell systems has to be taken into consideration in detailed studies of pathogenesis in lymphomas.

3. Immune Infiltrate Alterations in DLBCL

3.1. Role of Tregs. From a pathological point of view, tumor microenvironment and host immune suppression are most frequently associated with Tregs. Physiologically, they account for 4–10% of all peripheral CD4+ cells and are responsible for tolerance to autoantigens and inhibition or regulation of the immune response by suppression of effector cells. In malignancies, they affect tumor progression. These CD4+CD25+ cells intracellularly express the FOXP3 transcription factor, a Treg development regulator, and their essential marker in immunohistochemistry. They also
intracellularly express CD152 (CTLA4), also present on some CD4+ cells. A detailed assessment of their immunophenotype using flow cytometry [21] showed that FOXP3 and CTLA4 are expressed by up to 90% of CD4+CD25+ cells. Additionally, FOXP3 may, to a lesser extent, be also expressed in cytoplasm of CD4+CD25-cells. Following more detailed analysis, however, this paradox was explained by the fact that those were not CD25-cells but cells with low intensity of CD25 expression. Tregs have an immunosuppressive effect on CD4+CD25-cells of the immune response. They have the same effect on release of cytokines (IFNγ, IL-4) by these cells. This is mainly through IL-10 and TGFβ1 producing Tregs. The amount of Tregs is generally increased in non-Hodgkin lymphomas, as seen from the above study by Yang et al. showing a significant difference in the proportion of Tregs between biopsies from B-NHL patients and those from healthy controls. The reason for increased amounts of Tregs in NHL was explained by Han et al. who showed that malignant B cells obtained from patients with DLBCL and mantle cell lymphoma (MCL) in vitro induce conversion of CD4+CD25-T cells to CD4+CD25+ T cells, with acquisition of FOXP3 positivity [22]. However, the prognostic value of Tregs remains unclear, with individual studies on various types of lymphomas yielding different results. An immunohistochemistry study on the presence of Tregs in tumor tissue of 1,019 patients with DLBCL, follicular lymphoma (FL) or classical Hodgkin lymphoma (cHL) using microarrays concluded that Tregs are an important modulator of the microenvironment and their density in tumor tissue (in all the above entities) may contribute to prediction of prognosis [23]. Each type of tumors, however, has a different absolute count of Tregs. The authors point to an important moment in the lymphomagenesis and development of B cells, a reaction of the germinal center (GC), one of the most important points of B cell maturation. This is dependent on the presence of follicular helper T (Tfh) cells responsible for somatic hypermutation and immunoglobulin class switching. Tregs have been shown to suppress Tfh cells and thus maturation of B cells. According to the study, increased Treg counts positively influence survival in FL, GCB DLBCL, and cHL. Conversely, Tregs negatively influence survival in the non-GCB subtype which has different genesis than that of the GCB subtype, with the absolute Treg counts being identical in both variants. In their retrospective study of biopsy specimens from 96 DLBCL patients (both GCB and non-GCB), Lee et al. concluded that patients with higher Treg counts had significantly longer overall survival [24]. Once again, the prognosis was better in the GCB subtype. Another study conducted by a Wang and Ke provided classification of Tregs into 4 groups according to their roles: (a) suppressor Tregs, suppressing antitumor CD8+ T cells, are present in various types of solid tumors and lymphomas; (b) malignant Tregs, FOXP3-positive T cells in adult T-cell leukemia/lymphoma and cutaneous T-cell lymphoma, assuming the cells are malignant; (c) direct tumor-killing Tregs, killing tumor cells directly; and (d) incompetent Tregs, contributing to autoimmune symptoms in angioimmunoblastic T-cell lymphoma [25]. This new classification partly contributes to determine patients' prognosis, with suppressor or malignant Tregs, associated with decreased antitumor cytotoxicity and more favorable prognosis, being mostly prevalent in lymphoma patients. However, it is still not known whether this classification only reflects their function or whether individual Treg groups have different phenotypes as well.

3.2. Programmed Cell Death Ligand Pathway. A related immunomodulatory mechanism in the tumor microenvironment is interaction of the PD-1 receptor with its ligands, PD-L1 (syn. CD274, B7-H1) and PD-L2 (syn. CD273, B7-DC). PD-1 is a transmembrane protein present on activated T and B cells and macrophages, with mainly inhibitory function. Physiologically, it participates in regulation of autoimmune mechanisms. Its ligands have been known for approximately 10 years [26]. PD-L1 is physiologically expressed on antigen-presenting cells and has been shown to play an important role in antitumor immunity. As an important coinhibitor of host cell response is associated with patients’ poor response to therapy [27]. In cases of malignant diseases, it is frequently diagnosed on the tumor cell surface as one of mechanism used by these cells to ensure their resistance to cytotoxic activity of antitumor T cells. Moreover, this mechanism may result in induction of apoptosis in tumor-specific T cells. In hematological malignancies, variable expression of PD-L1 on cells of many lymphoma types has been reported. Conversely, PD-L2 expression is rather occasional, for example, in PMBCL [28]. Genes for both ligands are located in the 9p24.1 region, also associated with genes for JAK2. Studies on Hodgkin lymphoma cell lines (nodular sclerosis subtype) and PMBCL showed that JAK2 amplification increases transcription of PD-L1 [20]. In case of JAK2 inhibition, proliferation of cell lines of the two lymphoma types is decreased, providing another option for a therapeutic effect. As yet, only few studies have addressed expression of PD-1+ cells or PD-L1/PD-L2 with respect to prognosis of the disease. One of them is concerned with FL [29]. The authors studied the presence of PD-1+ T cells in tumor tissue using tissue microarrays with paraffin blocks of tissue samples obtained from 91 patients with newly diagnosed FL. PD1+ T cells were counted under standard conditions (1000x magnification, 9 visual fields from 3 follicles per sample). The results showed that the PD-1+ cell count correlated with the number of FOXP3+ Tregs, with PD-1+ T helper cells being established as an independent prognostic factor for patients’ overall survival.

3.3. MHC Class II Expression. Another way of how neoplasms may lower their immunogenicity is reduction of HLA antigens by decreasing expression of MHC class II proteins on antigen-presenting cells to CD4+ T cells, as shown in both DLBCL and PMBCL. Genetic studies revealed that one of the main factors responsible for transcription of all MHC class II genes is CIITA, encoded by the MHCG2TA gene (Wilkinson ST-12-4), with downregulation of MHC class II being correlated with decreased expression of the transactivator. CIITA gene fusions may also result in overexpression of the aforementioned PD-L1 and PD-L2 [18]. Roberts et al. used
gene expression profiling (GEP) and immunohistochemistry (IHC) to study expression of MHC class II and its regulation genes in patients with DLBCL and PMBCL in an attempt to explain the fact that while in PMBCL, frequent losses of MHC class II proteins had been reported, these patients paradoxically survived longer than those with DLBCL. The results showed that loss of MHC class II molecules is not characteristic for PMBCL and accounts for approximately 10% of cases in both PMBCL and non-GCB subtypes of DLBCL [30]. Results of GEP and IHC were different in earlier studies, with IHC studies reporting 5–40% of cases with loss of MHC class II proteins. This was reflected by the WHO classification definition, characterizing PMBCL as MHC class II negative. In this study, the IHC profile was determined by a single approach using an antibody against HLA-DR which seems to be most suitable for the diagnosis of MHC class II molecules. Pearson correlation showed that IHC and GEP results in this study were relatively consistent ($R = 0.68$). However, the authors concluded that GEP is a more sensitive method for detecting decreased expression of MHC class II molecules, given the variable detection threshold and tendency to overrating in IHC. Yet immunohistochemistry remains an important method in detection of MHC class II.

4. Clinical Impact of Tumor-Host Immune Interaction in DLBCL

The prognostic impact of some aspects of tumor-host immune interaction has been studied in the clinical setting. The most analyzed marker in DLBCL patients is HLA class II gene/protein expression intensity on lymphoma cells.

A follow-up study from the Leukemia and Lymphoma Molecular Profiling Project analyzed the effects of HLA-DR on survival and correlated gene expression with protein status and tumor-infiltrating lymphocytes. The 5-year overall survival was 24% in the lowest 10% of HLA-DR expression, 37% in the 10% to 25% group, 50% in the 25% to 50% group, and 55% for patients in the highest 50%. Further analysis demonstrated that the hazard ratio of death was a nonlinear function of HLA-DR expression [11].

In 2006, Roberts and coworkers published an extensive study comparing immunohistochemical detection of HLA-DR and relationship to gene expression [30]. Their data seemed to show that there could be a similar mechanisms of substantial and partial losses of MHC class II gene expression across the PMBCL, ABC, GCB, and unclassified subtypes of DLBCL. Kaplan-Meier plots of overall survival by average MHC class II gene expression in PMBCL were calculated. There was significantly better survival in cases with higher MHC class II expression; the survival increased significantly and incrementally with increasing expression ($P = 0.039$). In this study, immunohistochemical detection of HLA-DR was in close relationship to gene expression (Pearson correlation $R = 0.68$).

Rimsza et al. analyzed the impact of HLA II expression on a cohort of patients uniformly treated with MACOP-B regimen. In this study, the expression was analyzed using the tissue microarray method. The investigators found a frequent loss of HLA-DR expression (37% of cases) even after eliminating cases lacking staining of internal control cells. Patients lacking HLA-DR expression had a significantly inferior median survival of 4.2 years as compared to 16.2 years in HLA-DR expressors ($P = 0.037$). In multivariate Cox analysis, both IPI and HLA-DR were independent predictors of survival [31].

Key regulators of antitumor immunity and peripheral tolerance are CD4+ Tregs.

Numerous studies support the role of CD4+CD25+ Tregs in shaping the immune response to tumors. There is evidence that the cells restrain effective tumor immunosurveillance and promote tumor progression [32]. Therefore, it has been suggested that high Treg frequency may be closely correlated with poor survival rates in the majority of solid tumors [33]. In lymphomas, the situation is less clear. The prognostic value of these intratumoral Tregs varies according to the author, sample preparation, and DLBCL classification [4]. Hasselblom et al. reported that Tregs defined as FOXP3+ cells had no influence on the overall survival of 195 patients [34]. On the other hand, Lee et al. published a study of 96 DLBCL patients that confirmed the prognostic value of a higher number of FOXP3-positive T cells [24]. Remarkably, patients did not differ in clinical or laboratory parameters and distribution of FOXP3 cells was similar in GCB and non-GCB groups. The 5-year overall survival of patients with ≥2.3% of FOXP3-positive Tregs was 55%, as compared with only 25% for those with <2.3% of FOXP3-positive Tregs ($P = 0.003$). Tzankov et al. found higher density of Tregs to be associated with a good outcome in GCB DLBCL (32/55 patients) and with a poor outcome in non-GCB DLBCL (28/70 patients) [23].

These inconsistent results may be explained by many roles of Tregs in particular situations as described above. In patients with lymphomas where Tregs serve as tumor-killing Tregs and incompetent Tregs, antitumor cytotoxicity is enhanced. Thus, increased numbers of Tregs are associated with a good prognosis [25, 35].

Perspective prognostic factors with causal relationship with host immunity are recently published small deletions or point mutations of transcriptional regulator CIITA [12]. The functional consequences of CIITA gene fusions were found to be downregulation of surface HLA class II expression and overexpression of ligands of the programmed cell death-1 molecule (CD274/PD-L1 and CD273/PD-L2). Steidl et al. published a complex study where CIITA was proven to be a novel genetic mechanism underlying tumor-microenvironment interactions [18]. Patients with PMBCL treated with multiagent chemotherapy have significantly poorer disease-specific survival in the presence of a CIITA rearrangement ($P = 0.044$).

5. Conclusion

Recent years have brought novel insights into the network of interactions between lymphoma cells and host immune defense. Novel cytogenetic aberrations have an apparent effect on tumor immunogenicity and T-cell suppression. Standardization of tissue microarray methods has laid the
foundations for finding novel immune-related prognostic factors such as HLA-DR expression or the PD ligand/receptor pathway [36, 37]. Although the mechanisms underlying various roles of Tregs in patients with lymphomas remain unknown, in some cases they have a strong predictive potential. Therefore, further research is needed in this regard as well as on the use of Tregs as prognostic factors.

Finally, there are very new drugs which can target pathways of immune dysfunction. Selective JAK2 inhibitor is now in clinical testing [38], and very recently, a study of pathways of immune dysfunction. Selective JAK2 inhibitor potential. Therefore, further research is needed in this regard remaining various roles of Tregs in patients with lymphomas.

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References


[22] Y. Han, J. Wu, L. Bi et al., “Malignant B cells induce the conversion of CD4+CD25− T cells to regulatory T cells in
B-cell non-Hodgkin lymphoma,” *PLoS ONE*, vol. 6, no. 12, Article ID e28649, 2011.


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