EUROPEAN HEMATOLOGY ASSOCIATION

Immunotherapy in lymphoma - Section 1

The role of the microenvironment in the pathogenesis of B-cell lymphomas

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Take-home messages

- The tumor microenvironment plays a critical role in the biology of B-cell malignancies.
- Composition of the tumor microenvironment can impact the clinical course.

Introduction

In the past decades, significant advances in the understanding of the biology of various subtypes of malignant B-cell lymphomas have been made. Numerous studies identified, in the malignant lymphoma cells, different genetic aberrations and dysregulated oncogenic signaling pathways that contribute to the molecular pathogenesis of these entities. However, additionally the importance of the non-malignant cells surrounding the neoplastic B-cells, the so-called tumor microenvironment, became evident. Furthermore, various studies showed that the composition of the microenvironment can impact the clinical course of affected patients further underscoring its importance. Thus, a better understanding of the role of the tumor microenvironment is crucial. Within this review, we summarize the current knowledge of the microenvironment for three B-cell lymphoma subtypes.

Diffuse large B-cell lymphoma

Accounting for roughly 30-40% of all lymphoma cases, diffuse large B-cell lymphoma (DLBCL) represents the most common malignant lymphoma subtype. Gene expression analyses identified two major molecular subtypes, termed activated B-cell like (ABC) and germinal center B-cell like (GCB) DLBCL.[1] Diverse genetic aberrations contributing to either ABC or GCB DLBCL pathogenesis have been unraveled. It has been shown that some of these abnormalities can also influence patients' response to standard of care therapy.[1] However, additionally it became evident that besides genetic features of the lymphoma cells that the microenvironment can dictate prognosis of affected patients. Gene expression profiling identified two signatures called 'stromal-1' and 'stromal-2' that reflect expression patterns of non-malignant cells within the DLBCL microenvironment.[2] The 'stromal-1' signature includes genes that are expressed in normal mesenchymal tissues, many of which encode proteins of the extracellular matrix as well as genes normally expressed in monocytes. This signature was associated with favorable outcome, whereas the so called 'stromal-2' signature that comprised genes expressed in endothelial cells or genes encoding important regulators of angiogenesis, was associated with adverse survival.[2]

However, the specific contribution of individual microenvironmental components on DLBCL prognosis is still not completely understood and data from various studies are even contradictory (Table 1). Especially the role of tumor-associated macrophages and regulatory T-cells (T_{reg}) has been analyzed recently, but could not reveal uniform results. High numbers of macrophages as measured by CD68 staining were found to be a favorable prognostic marker for DLBCLs treated with immunochemotherapy.[3,4] In contrast, high numbers of CD68+ cells indicated poor prognosis for patients treated without rituximab.[4] Furthermore, an increased ratio of CD68/CD163 double positive cells was correlated with adverse survival.[3] Similarly controversial results have been obtained for the prognostic impact of T_{reg}s. Whereas several studies suggested that a high number of T_{reg} cells is correlated with favorable outcome in DLBCL,[3,5,6] one study reported a positive influence of high T_{reg}s on survival only in GCB DLBCLs and a negative prognostic effect in non-GCB DLBCLs.[7] Closely related to the tumor microenvironment is also the ability of tumor cells to evade the immune response of the host. Mechanisms supporting the immune evasion in DLBCL include loss of B2M and CD58 expression to prevent recognition by circulating cytotoxic T-cells and natural killer (NK) cells.[8] Furthermore, the PD-1/PD-L1 pathway can promote immune evasion. In DLBCLs an increased PD-L1 expression on tumor cells was associated with the ABC DLBCL subtype.[9] High expression seems to be due to genetic alterations affecting the *PD-L1*

locus. These alterations are more frequent in ABC DLBCLs leading to an increased expression of PD-L1.[10]

Follicular lymphoma

An important role in the biology has also been established for the tumor microenvironment in follicular lymphoma (FL). Gene expression profiling data revealed two gene expression signatures derived from non-malignant cells that were correlated with prognosis of affected patients.[11] The first signature termed 'immune-response 1 signature' is enriched for genes typically expressed in T-cells and is correlated with favorable outcome. In contrast, the 'immune-response 2 signature' that comprised genes that are expressed in macrophages and dendritic cells, is associated with poor prognosis.[11]

The prognostic impact of individual microenvironmental components has been the focus of several studies (Table 1). For CD8+ cytotoxic T-cells it was shown that increased numbers of cells with high granzyme B expression levels are correlated with a longer progression-free survival, whereas the number of CD8+ cells alone did not correlate with survival.[12] Another study implicated that the numbers of a CD8+ subset with low PD-1 expression correlated with shorter survival.[13]

The impact of macrophages on FL survival has also been controversial. Whereas some studies implicated high numbers of tumor associated macrophages to be correlated with adverse outcome in FL patients, other analyses suggested favorable outcome.[14-16] Potentially, these divergent results are related to patient treatments. PD-1 expression potentially contributing to immune evasion via the PD-1/PD-L1 pathway has also been studied, but again the prognostic implications are not fully elucidated.[17,18]

Classical Hodgkin lymphoma

Classical Hodgkin lymphoma (cHL) is unique with respect to its histopathological appearance, as the malignant Hodgkin Reed-Sternberg (HRS) cells represent only the minority of cells that are surrounded by an extensive number of non-malignant immune cells constituting the tumor microenvironment. As for DLBCL and FL different microenvironmental factors contribute to cHL prognosis (Table 1). Increased numbers of CD68+ macrophages were associated with inferior survival.[19,20] In contrast, high numbers of non-malignant CD20-positive B-cells were correlated with favorable prognosis.[20,21] Similarly, increased numbers of T_{reg} cells pointed towards a superior outcome.[20] In contrast, a low ratio of T_{reg} to cytotoxic T-cells/NK cells correlated with poor survival.[22] The ability of HRS cells to evade the immune response of the host seems to be mediated through different molecular mechanisms. Besides loss of MHCI and MHCII expression due to inactivating *B2M* mutations or translocations involving the *CIITA* gene,[23,24] HRS cells are also characterized by high expression of PD-L1 and/or PD-L2 due to chromosomal amplifications affecting 9p24 resulting in binding to PD-1 positive T-cells and subsequent T-cell exhaustion most likely explaining the high efficacy of checkpoint inhibitors in relapsed/refractory cHL patients.[25-27]

In summary, the microenvironment plays an important role in the pathogenesis of different B-cell malignancies. However, the exact contribution of individual components needs to be addressed and defined more precisely in future studies. Deciphering the exact role of the tumor microenvironment in the biology of these entities might lead to more specific and potentially less toxic treatment regimens.

Table 1. Prognostic impact of microenvironmental factors in B-cell lymphoma.

Lymphoma subtype	Microenvironmental factor	Prognostic impact
DLBCL	Macrophages	High numbers of macrophages associated with favorable outcome when treated with immunochemotherapy[3,4] High numbers of macrophages associated with adverse outcome when treated without rituximab[3,4]
	T_{reg} cells	High numbers of T_{reg} cells correlated with favorable outcome in GCB DLBCL/DLBCL[3,5-7] High numbers of T_{reg} cells correlated with adverse outcome in non-GCB DLBCL[7]
Follicular lymphoma	Cytotoxic T-cells	High numbers of granzyme B+ cells correlated with favorable outcome[12] Numbers of CD8+ cells with low PD-1 expression correlated with shorter survival[13]
	Macrophages	Impact of numbers of macrophages on FL survival controversial; some studies implicated high numbers of tumor associated macrophages to be correlated with adverse outcome in FL patients, other analyses suggested favorable outcome[14-16]
Classical Hodgkin lymphoma	Macrophages	Increased numbers correlated with inferior survival[19,20]
	Non-malignant B-cells	Increased numbers correlated with favorable outcome[20,21]

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References

1. Nogai H, Dorken B, Lenz G. Pathogenesis of Non-Hodgkin's Lymphoma. J Clin Oncol 2011;29:1803-11.
*2. Lenz G, Wright G, Dave SS, et al. Stromal gene signatures in large-B-cell lymphomas. N Engl J Med 2008;359:2313-23.

This reference highlights the prognositc impact of the tumor microenvironment for DLBCL survival.

3. Nam SJ, Go H, Paik JH, et al. An increase of M2 macrophages predicts poor prognosis in patients with diffuse large B-cell lymphoma treated with rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone. Leuk Lymphoma 2014;55:2466-76.
4. Riihijarvi S, Fiskvik I, Taskinen M, et al. Prognostic influence of macrophages in patients with diffuse large B-cell lymphoma: a correlative study from a Nordic phase II trial. Haematologica 2015;100:238-45.
5. Lee NR, Song EK, Jang KY, et al. Prognostic impact of tumor infiltrating FOXP3 positive regulatory T cells in diffuse large B-cell lymphoma at diagnosis. Leuk Lymphoma 2008;49:247-56.
6. Coutinho R, Clear AJ, Mazzola E, et al. Revisiting the immune microenvironment of diffuse large B-cell lymphoma using a tissue microarray and immunohistochemistry: robust semi-automated analysis reveals CD3 and FoxP3 as potential predictors of response to R-CHOP. Haematologica 2015;100:363-9.
7. Tzankov A, Meier C, Hirschmann P, et al. Correlation of high numbers of intratumoral FOXP3+ regulatory T cells with improved survival in germinal center-like diffuse large B-cell lymphoma, follicular lymphoma and classical Hodgkin's lymphoma. Haematologica 2008;93:193-200.
8. Challa-Malladi M, Lieu YK, Califano O, et al. Combined genetic inactivation of beta2-Microglobulin and CD58 reveals frequent escape from immune recognition in diffuse large B cell lymphoma. Cancer cell 2011;20:728-40.
9. Kiyasu J, Miyoshi H, Hirata A, et al. Expression of programmed cell death ligand 1 is associated with poor overall survival in patients with diffuse large B-cell lymphoma. Blood 2015; 126:2193-2201.
10. Georgiou K, Chen L, Berglund M, et al. Genetic basis of PD-L1 overexpression in diffuse large B-cell lymphomas. Blood 2016;127:3026-34.
*11. Dave SS, Wright G, Tan B, et al. Prediction of survival in follicular lymphoma based on molecular features of tumor-infiltrating immune cells. N Engl J Med 2004;351:2159-2169.

This paper highlights the role of the tumor microenvironment for the outcome of FL patients.

12. Laurent C, Muller S, Do C, et al. Distribution, function, and prognostic value of cytotoxic T lymphocytes in follicular lymphoma: a 3-D tissue-imaging study. Blood 2011;118: 5371-9.
13. Yang ZZ, Grote DM, Ziesmer SC, et al. PD-1 expression defines two distinct T-cell sub-populations in follicular lymphoma that differentially impact patient survival. Blood Canc J 2015;5:e281.
14. Farinha P, Masoudi H, Skinnider BF, et al. Analysis of multiple biomarkers shows that lymphoma-associated macrophage (LAM) content is an independent predictor of survival in follicular lymphoma (FL). Blood 2005;106:2169-74.
15. Taskinen M, Karjalainen-Lindsberg ML, Nyman H, et al. A high tumor-associated macrophage content predicts favorable outcome in follicular lymphoma patients treated with rituximab and cyclophosphamide-doxorubicin-vincristine-prednisone. Clin Cancer Res 2007;13:5784-9.
16. Canioni D, Salles G, Mounier N, et al. High numbers of tumor-associated macrophages have an adverse prognostic value that can be circumvented by rituximab in patients with follicular lymphoma enrolled onto the GELA-GOELAMS FL-2000 trial. J Clin Oncol 2008;26:440-6.
17. Carreras J, Lopez-Guillermo A, Roncador G, et al. High numbers of tumor-infiltrating programmed cell death 1-positive regulatory lymphocytes are associated with improved overall survival in follicular lymphoma. J Clin Oncol 2009;27:1470-6.
18. Richendollar BG, Pohlman B, Elson P, Hsi ED. Follicular programmed death 1-positive lymphocytes in the tumor microenvironment are an independent prognostic factor in follicular lymphoma. Human pathology 2011;42:552-7.
*19. Steidl C, Lee T, Shah SP, et al. Tumor-associated macrophages and survival in classic Hodgkin's lymphoma. N Engl J Med 2010;362:875-85.

This reference describes how macrophages influence the survival of patients with Hodgkin lymphoma.

20. Greaves P, Clear A, Coutinho R, et al. Expression of FOXP3, CD68, and CD20 at diagnosis in the microenvironment of classical Hodgkin lymphoma is predictive of outcome. J Clin Oncol 2013;31:256-62.
21. Panico L, Tenneriello V, Ronconi F, et al. High CD20+ background cells predict a favorable outcome in classical Hodgkin lymphoma and antagonize CD68+ macrophages. Leuk Lymphoma 2015;56: 636-42.
22. Kelley TW, Pohlman B, Elson P, Hsi ED. The ratio of FOXP3+ regulatory T cells to granzyme B+ cytotoxic T/NK cells predicts prognosis in classical Hodgkin lymphoma and is independent of bcl-2 and MAL expression. Am J Clin Pathol 2007;128:958-65.
23. Reichel J, Chadburn A, Rubinstein PG, et al. Flow sorting and exome sequencing reveal the oncogenome of primary Hodgkin and Reed-Sternberg cells. Blood 2015;125:1061-72.
*24. Steidl C, Shah SP, Woolcock BW, et al. MHC class II transactivator CIITA is a recurrent gene fusion partner in lymphoid cancers. Nature 2011;471:377-81.

This reference reveals an important mechanism contributing to immune evasion in Hodgkin lymphoma.

25. Green MR, Monti S, Rodig SJ, et al. Integrative analysis reveals selective 9p24.1 amplification, increased PD-1 ligand expression, and further induction via JAK2 in nodular sclerosing Hodgkin lymphoma and primary mediastinal large B-cell lymphoma. Blood 2010;116:3268-77.
26. Chen BJ, Chapuy B, Ouyang J, et al. PD-L1 expression is characteristic of a subset of aggressive B-cell lymphomas and virus-associated malignancies. Clin Cancer Res 2013;19:3462-73.
27. Ansell SM, Lesokhin AM, Borrello I, et al. PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. N Engl J Med 2015;372:311-9.