The Biological Environment of Hodgkin's Lymphoma and the Role of the Chemokine CCL17/TARC

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Abstract: Hodgkin's lymphoma is a lymphoproliferative disease, which differs in its morphology and therapeutic response from other lymphomas. Neoplastic cells represent only a minor cell population of the tumour, while the major part of the tumour is formed by inflammatory cells. It results from the production of cytokines and chemokines both by neoplastic cells and by inflammatory cells. An important prognostic marker in Hodgkin's lymphoma appears to be the chemokine (C-C motif) ligand 17 (CCL17), also known as thymus and activation-related chemokine (TARC). This chemokine is expressed by many cell types and tissues, and in the case of Hodgkin lymphoma, also by Reed-Sternberg cells. CCL17/TARC binds to chemokine receptors CCR4 and CCR8 and displays chemotactic activity for T lymphocytes and some other leukocytes. The understanding of biological pathways in Hodgkin's lymphoma could be important for monitoring of disease activity and for the development of future targeted therapy.

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Introduction

It has been recently recognized that Hodgkin's lymphoma includes two disease entities, which differ in clinical and biological features: classical Hodgkin's lymphoma and nodular lymphocyte predominant Hodgkin's lymphoma.

The histological diagnosis of classical Hodgkin's lymphoma is based on the identification of Reed-Sternberg cells in the inflammatory cellular background. The diagnosis of nodular lymphocyte predominant Hodgkin's lymphoma is based on the identification of multilobated lymphocyte and histiocyte blasts, called L&H cells. Both entities are characterized by the low percentage of neoplastic cells within the inflammatory cells. This is the result of the action of chemotactic cytokines that are produced by both neoplastic and inflammatory cells. The chemokine CCL17/TARC seems to play a crucial role in the formation of tumour inflammatory background.

Classical Hodgkin's lymphoma

Classical Hodgkin's lymphoma is characterized by the presence of Reed-Sternberg cells and their variants. Histologically, Reed-Sternberg cells contain a large polyploid nucleus, or they are multinucleated. Each lobe or each nucleus of multinucleated Reed-Sternberg cells contains one eosinophilic nucleolus. Other types of neoplastic cells observed in the tumour are the Hodgkin cells (non-lobated mononuclear cells), the "lacunar" cells (mononuclear or multinucleated) and the "zombie" cells (apoptotic Hodgkin cells). With respect to the relationship between the mononucleated Hodgkin and multinucleated Reed-Sternberg cells, there is evidence that Reed-Sternberg cells develop from Hodgkin cells by the process of endomitosis [1, 2].

The origin of the Hodgkin/Reed-Sternberg (HRS) cells has been clarified by a single-cell polymerase chain reaction method used to analyze B-cell receptor (BCR) or T cell receptor (TCR) gene rearrangements [3]. HRS cells carry clonal BCR/immunoglobulin gene rearrangements, which supports their B-cell lineage origin. Nearly all cases carry somatically mutated immunoglobulin variable (V) gene rearrangements suggesting that these cells are derived from germinal-center or postgerminal-center B cells [4, 5, 6, 7]. These mutations are found physiologically in antigen-activated B cells, the process leading to the mutation is called "somatic hypermutation" [8]. Some cases of classical Hodgkin's lymphoma carry "crippling" somatic mutations, such as nonsense mutations and deletion, which is why transcription cannot take place. The majority of the germinal-center B-cells does not survive these "crippling mutations" and they undergo apoptosis. Therefore, some additional mechanisms that would rescue at least some of these cells from apoptosis must exist. It may be concluded that HRS cells carrying the destructive mutations are derived from preapoptotic germinal-center B-cells that escaped apoptosis [9, 10, 11, 12].

Another cause of apoptotic death of germinal-center B-cells may be the unfavourable replacement mutations impairing the proper folding of the antibody

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variable domain or heavy- and light-chain pairing. The pattern of somatic mutation in rearranged immunoglobulin genes of HRS cells indicates that these cells are derived from the pool of preapoptotic "crippled" germinal-center B-cells. However, HRS cells can survive, although they lack the BCR. The reason for the rescue of the HRS cell precursors from apoptosis is still unclear. However, it appears that it may involve Epstein-Barr virus infection (EBV).

EBV plays an important role in pathogenesis in Hodgkin lymphoma and almost fifty-percent of classical Hodgkin lymphoma is EBV positive [13]. The EBV infected HRS cells express viral proteins, the EBV nuclear antigen 1 (EBNA1) and the latent membrane proteins LMP1, LMP2a. The latent membrane protein LMP2a affects BCR signalling. The specific phosphotyrosine cytoplasmic motifs within the N-terminal tail domain of LMP2a alter normal BCR signal transduction in B cells by reducing levels of tyrosine kinase Lyn and by blocking tyrosine phosphorylation and calcium mobilization following BCR cross-linking [14, 15]. The capability of LMP2a to replace the function of BCR, which is highly important for survival signals, may rescue germinal-centre B-cells with unfavourable somatic mutations in their BCR from apoptosis [16, 17, 18]. This means that B-cells with these unfavourable somatic BCR hypermutations can survive and become a precursor of HRS tumour clones, only if they are EBV positive [19, 20]. However, in the established HRS cell clone, which lost its B-cell phenotype, the role of LMP2a is less important. The HRS cells are not dependent on the BCR or LMP2a signalling. This finding indicates that EBV infection is an early event in the development of Hodgkin lymphoma [19].

In some cases, the classical Hodgkin lymphoma HRS cells express some markers typically associated with T-cells. HRS cells may be seemingly derived from T cells, as it is suggested by the clonal TCR gene rearrangements. On the other hand, some HRS cells with partial T-cell phenotype carry rearranged BCR/immunoglobulin V genes, which mean that T cell markers can be aberrantly expressed by B-lineage-derived HRS cells [21, 22].

Nodular lymphocyte predominant Hodgkin's lymphoma
This type of Hodgkin's lymphoma is characterized by a mixture of lymphocytes and epitheloid histiocytes (L&H cells). These cells are called "popcorn cells" because of the specific multilobated nuclear morphology. L&H cells are large and have lobulated nuclei and small to moderate-sized basophilic nucleoli, often adjacent to the nuclear membrane; the cytoplasm is basophilic.

Immunohistological examination showed that L&H cells are characterized by the constant absence of CD30 and CD15 and they consistently carry B-cell antigens by the consistent expression of CD20, J chain, and CD79a. On the other hand, HRS cells are characterized by the constant expression of CD30, frequent expression of CD15, and the absence of J chain [23, 24]. The L&H cells in lymphocyte-predominant Hodgkin's lymphoma lack crippling mutations, express a BCR, and often show intraclonal V gene diversity explained by hypermutation activity during

clonal expansion. L&H cells appear to derive from selected, mutating germinal center B-cells [25, 26].

The inflammatory background
The majority of Hodgkin's lymphoma tissue consists of inflammatory non-malignant cells. In classical Hodgkin's lymphoma, a mixture of cell types can be found, usually with predominant T lymphocytes, but eosinophils, neutrophils, histiocytes, plasma cells, mast cells and fibroblasts are present as well. In nodular lymphocyte-predominant Hodgkin lymphoma, neutrophils and eosinophils are rare; plasma cells are uncommon and they are observed only between follicles.

The inflammatory cells are attracted by cytokines and chemokines secreted by HRS cells. On the other hand, inflammatory cells produce many factors supporting the growth and survival of the Hodgkin and Reed-Sternberg cells and increased collagen synthesis [27, 28]. One of the crucial chemokines produced by Reed-Sternberg cells, supporting the influx of Th-2 lymphocytes into the inflammatory background in classical Hodgkin's lymphoma, is the chemokine CCL17/TARC.

CCL17/TARC and its role in inflammatory background
CCL17/TARC is a protein that was identified by cloning the D3A gene from peripheral blood mononuclear cells (PBMCs) after stimulation with phytohaemagglutinin [29]. It has a predicted mass of 8 kDa and shares 25–30% sequence homology with other known members of the C-C chemokine family, including full conservation of the four cysteine residues that are characteristic of chemokines. The other major source of CCL17/TARC expression was identified in the thymus, which resulted in the protein name "Thymus and Activation-Related Chemokine" (TARC) [29].

TARC binds to chemokine receptors CCR4 and CCR8. This chemokine plays important roles in T cell development in thymus as well as in the trafficking and activation of mature T cells. The CCR4 is high affinity functional receptor for TARC and is mainly expressed on T cells (largely on CD4+ T cells) and is not activated by other chemokines. Other types of leukocytes, B cells and monocytes, may also express CCR4 but only at low levels [30].

The CCR8 belongs to the transmembrane-spanning receptor family and responds to TARC and some other chemokines [31]. It is expressed on lymphoid tissues, i.e. thymus, spleen and lymph nodes and is abundantly up-regulated in Th2 lymphocytes, which suggest its possible role in Th2 lymphocyte activation, migration and differentiation [32]. In Hodgkin's disease, TARC is highly expressed by Reed-Sternberg cells. The expression of TARC was not observed in L&H cells from nodular lymphocyte predominant Hodgkin's lymphoma, but its production is irregularly detected in T-cell rich B-cell lymphoma. High expression of CCL17 by HRS cells might explain the influx of lymphocytes with Th2-like phenotype into their proximity, creating a favourable environment for survival of HRC cells. On the

other hand, Th2 type cytokines (IL-4 and IL-13) stimulate the production of TARC by HRC cells [33, 34]. Several studies have analyzed serum levels of cytokines and chemokines in patients with Hodgkin lymphoma in comparison to levels observed in controls. In a retrospective study by the German Hodgkin study group, TARC serum levels were found to be elevated in 90% of primary Hodgkin's disease patients. The TARC levels in serum samples prior and post therapy of patients and control groups were measured by ELISA. A TARC level of > 500 pg/ml was considered high. The retrospective study showed that TARC serum levels at diagnosis correlated with stage, erythrocyte sedimentation rate, leukocyte counts, and lymphocyte counts. A TARC serum level > 2,000 pg/ml following treatment was found to correlate with a poor overall survival. TARC serum levels also correlated with tumour load, and levels dropped after therapy [35].

Conclusion

Hodgkin's lymphoma is a type of lymphoproliferative disease, although it differs from other non-Hodgkin lymphomas, both clinically and pathologically. The main feature of classical Hodgkin's lymphoma is that only 1% of the tumour bulk consists of neoplastic cells – the rest is made up of inflammatory background, which is formed mainly by small lymphocytes, neutrophils, eosinophils, plasma cells, mast cells, and some other cell types. This typical biological environment is the result of autocrine and paracrine interactions between neoplastic cells and reactive elements by means of several cytokines and chemokines. This leads to the formation of a favourable environment for neoplastic cells, in which they proliferate and can escape from apoptosis. The Hodgkin/Reed-Sternberg cells originate in B-cell lineage and they are derived from preapoptotic germinal center B-cells that escaped apoptosis. The reason for the rescue from apoptosis may lie in Epstein-Barr virus infection (EBV). One of the most recent detectable serum markers in classical Hodgkin lymphoma seems to be the chemokine TARC, which can be used to monitor disease activity in Hodgkin lymphoma and adds valuable information on therapy success.

It is important to understand the biological background of Hodgkin's lymphoma. Knowledge of the specific pathways can be useful in the future designing of targeted therapy and in monitoring of the activity of the baseline disease.

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