Pathology – Research and Practice 211 (2015) 901–904

Contents lists available at ScienceDirect

Pathology – Research and Practice

journal homepage: www.elsevier.com/locate/prp

Review

From a pathologist's point of view: Histiocytic cells in Hodgkin lymphoma and T cell/histiocyte rich large B cell lymphoma

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ARTICLE INFO

Keywords:
Macrophages
Reactive bystander cells
Hodgkin lymphoma
T cell/histiocyte rich large B cell lymphoma
Metallothionein 2A

ABSTRACT

While tumor cells were the focus of research for many years, only recently have attempts been made to understand the role of the reactive bystander cells in malignant lymphomas. In certain types of lymphomas, such as Hodgkin lymphoma and T cell/histiocyte rich large B cell lymphoma, more than 90% of the infiltrate represent non-neoplastic cells, and these have important functions for the development and progression of the tumor. Among the bystander cells are histiocytes of particular importance, which vary largely in number, shape and quality among different patients. In the present review, recent findings on the prognostic impact of histiocytic bystander cells in these lymphomas, as well as their molecular characteristics, are discussed. A better understanding of the role of these histiocytes may provide new concepts for diagnosis and treatment.

Contents

1. Introduction

For many years, lymphoma pathology has focused on the main players in these tumors, the malignant tumor cells. Different efforts have been made to classify lymphomas according to their growth patterns or the cytology of the tumor cells [1,2]. However, not only the tumor cells, but also the microenvironment contributes significantly to the malignant phenotype observed. Whereas in some lymphomas this microenvironment is so abundant that it has been included as part of the diagnosis [3], in other entities, such as diffuse large B cell lymphoma (DLBCL) and follicular lymphoma, the importance of the reactive bystander cells has only recently been recognized [4,5]. For several lymphoma entities, it was not possible to establish cell lines due to the strong dependency of the tumor cells on the reactive bystander cells, which were not viable under cell culture conditions. Therefore, malignant lymphomas can be divided into more and less microenvironment-dependent types. Lymphomas which are strongly dependent on their microenvironment are e.g. follicular lymphoma, marginal zone lymphoma, Hodgkin lymphoma (HL), T cell/histiocyte rich large B cell lymphoma, as well as angioimmunoblastic or peripheral T cell lymphoma. Surprisingly, both B- and T cell neoplasias can equally depend on their microenvironment. In contrast, Burkitt lymphoma and some cases of DLBCL are rather independent of their microenvironment, which is also reflected by the usually low content of bystander cells in the lymphoma infiltrate and the availability of a large number of corresponding cell lines. HL represents the most extreme relationship between tumor cell content (frequently <1% of the infiltrate) and reactive bystander cells. Surprisingly, HL cell lines could be established which grow in culture without the respective bystander cells. However, these were established from relapsed or refractory patients and frequently from pleural effusions, in which the tumor cells had already gained independency from their environment and were able to grow autonomously in suspension [6–8].

The reactive microenvironment of HL usually contains a huge spectrum of cells, ranging from non-neoplastic B cells and special T cell subsets to dendritic cells, macrophages, mast cells, eosinophils

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http://dx.doi.org/10.1016/j.prp.2015.10.012
0344-0338/

and stromal cells. The present review will focus on histiocytic cell types in the reactive microenvironment.

2. Macrophage subtypes

While pathologists described different types of histiocytes a long time ago, histiocytic surface markers have only recently been characterized by immunologists. Pathologists define macrophages by their shape and phagocytic function, as well as epithelioid cells, which primarily secrete cytokines. In contrast to this morphologic description, immunologists classify all macrophages in connective tissue as histiocytes or tissue macrophages [9]. One of the most important features of tissue macrophages is the take up and presentation of antigens [10]. Originally, two subsets of classically activated proinflammatory (M1) and alternatively activated (M2) macrophages have been described [11,12] which participate in Th1 and Th2 triggered immune responses, respectively. Morphologically recognizable histiocyte subsets have so far not been well characterized by surface markers. However, there are certain reactions like tuberculoid granulomas, which have been shown to express inducible nitrogen oxide (NO) synthetase [13], corresponding to M1-polarized macrophages. Secretion of NO and reactive oxygen species (ROS) is involved in tissue injury and necrosis as it is typically observed in caseous necrosis in the center of tuberculoid granulomas, consistent with a M1 phenotype of these epithelioid cells. With reference to the complex situation in vivo, Qian and Pollard [14] described six types of macrophages, the activated and the angiogenic type, but also an invasive, metastasis-associated, perivascular and immunosuppressive subtype. These different types secrete unique, but also partly overlapping cytokines. The invasive type of macrophages is found at the outer borders of solid tumors, since this represents the localization where malignant tumors grow fastest. These invasive macrophages were characterized in mice to have an active Wnt signaling [15]. Whereas it was previously hypothesized that all tissue macrophages derive from circulating peripheral blood monocytes [16], recent studies have shown that mature macrophages can proliferate in the tissue in response to specific stimuli without loss of functional differentiation [17,18]. These tissue-resident and self-renewing macrophages were shown to have an M2-like phenotype, whereas monocyte-derived macrophages, which are recruited to the tissue during an inflammatory response, are supposed to be M1-polarized [19].

3. Macrophages in Hodgkin lymphoma and T cell/histiocyte rich large B cell lymphoma

In 2010, the important impact of macrophages in the microenvironment of classical HL on event free and overall survival was first remarked [20]. Many other studies conducted since then have examined the impact of macrophages on clinical behavior. Whereas some could confirm previous observations and found a correlation between a high number of macrophages, mixed cellularity subtype and Epstein–Barr virus (EBV) infection of the Hodgkin- and Reed–Sternberg (HRS) cells [21–23], others could not confirm the prognostic impact of a high number of tissue macrophages [24,25]. However, the methods how the macrophage content was counted (CD68- versus CD163-staining) and the thresholds differed between different studies and so far no general applicable test is available. A whole tissue gene expression study identified a 23-gene outcome predictor, which included several macrophage- and M1-related genes like CXCL10, STAT1, IFNG, LYZ, CD68 and CD14 [26]. It may therefore be easier and more precise to quantify macrophages according to specific transcripts than counting numbers of macrophages. Particularly, the presence of special subtypes of polarized macrophages can better be assessed by the quantification of different transcripts than by pure morphology.

HIV-positive individuals with HL take a special position concerning the macrophage content in the HL infiltrate: they can present a particularly high macrophage content in the HL microenvironment when the CD4 count in the peripheral blood is low [27]. Therefore, the extent of macrophage infiltration in the lymphoma tissue seems to be related to the availability of CD4 T cells and monocytes in the blood, and monocytes/macrophages can replace CD4 T cells under certain conditions [27]. CD4 T cells get attracted to the microenvironment of HL by several cytokines like TARC and RANTES [28], which are secreted by the HRS cells. In HIV patients, CD4 blood counts are regularly tested, and the development of HL can therefore well be monitored in these patients. Already one year before HL becomes manifest, CD4 cells start to decrease in the peripheral blood, indicating recruitment of CD4 cells to the lymph nodes with HL infiltrates [29].

Interestingly, not only a high content of macrophages in the lymph nodes affected by HL was related to a worse clinical outcome: also a low ratio between lymphocyte and monocyte count in the peripheral blood was related with an adverse prognosis in classical and nodular lymphocyte predominant HL (in HIV-negative individuals) [30,31]. Therefore, in contrast to recent findings indicating that macrophages can renew in the tissue [17], the reactive HL microenvironment in the lymph node seems to reflect the availability of different cell types in the peripheral blood.

The molecular mechanisms how macrophages contribute to the dissemination and advanced stage of classical HL have only allusively been characterized. Applying only a limited number of immunohistochemical markers macrophage polarization cannot definitely be determined. CD163, which was originally described as M2 marker, is e.g. negative in all forms of epithelioid cells (Fig. 1), which are particularly found in mixed cellularity subtype and nodular lymphocyte predominant HL (NLPHL), although epithelioid cells can express other M2-markers [32]. In contrast, most subsets of macrophages express CD163 and even classically (M1) activated macrophages can be CD163-positive [33]. The information obtained by approaches, applying a restricted set of immunostainings is therefore very limited and the complexity of macrophage subsets cannot be sufficiently subdivided in the respective types [34].

Furthermore it is unclear in how far the molecular characterized macrophage subsets can be translated into the morphological variety of histiocytic cells as can be demonstrated in histologic slides. This is mainly due to the difficulties which are encountered by the isolation of the cells by laser microdissection and the availability of frozen tissue. In gene expression profiling, laser microdissected spindle shaped epithelioid cells from granulomas of sarcoidosis, [32] showed an enrichment of the M1 signature described by Martinez et al. [11], comparable to tuberculoid granulomas. However, all other types of epithelioid cells tested (round epithelioid cells from Piringer lymphadenitis as well as NLPHL) expressed both M1- and M2-related genes (Fig. 2). This confirms that the gene expression profiles of histiocytes are more complex than the division into M1 and M2 subtypes.

In T cell/histiocyte rich large B cell lymphoma usually a high content of histiocytes is encountered in the infiltrate. However, to date it is unclear why such high numbers of histiocytic cells accumulate in this tumor. Gene expression profiling revealed a strong expression of metal-binding proteins like Metallothionein 2A (MT2A) [32], which can scavenge ROS [35] and which can be induced by oxidative stress [36]. The presence of this particular type of MT2A-positive macrophages is to our knowledge restricted to this lymphoma and is additionally only found in T cell/histiocyte rich large B cell lymphoma-like variants of NLPHL. Epithelioid cells in typical NLPHL, as well as other forms of classical HL and reactive lesions, are usually MT2A-negative or only present a weak or