The role of T cells in the microenvironment of Hodgkin lymphoma

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ABSTRACT
The cellular microenvironment in HL is dominated by a mixed infiltrate of inflammatory cells with typically only 1% or a few percent of HRS tumor cells. HRS cells orchestrate this infiltrate by the secretion of a multitude of chemokines. T cells are usually the largest population of cells in the HL tissue, encompassing Th cells, TregS, and CTLs. Th cells and TregS presumably provide essential survival signals for the HRS cells, and the TregS also play an important role in rescuing HRS cells from an attack by CTLs and NK cells. The interference with this complex interplay of HRS cells with other immune cells in the microenvironment may provide novel strategies for targeted immunotherapies. J. Leukoc. Biol. 99: 000–000; 2016.

Introduction
With an incidence of 3–4 new cases per year per 100,000 persons, HL is one of the most frequent lymphomas in the Western world. Today, ~80–90% of HL patients can be cured [1]. cHL, with its subtypes of nodular sclerosis, mixed cellularity, and lymphocyte-rich and -depleted HL, accounts for ~95% of cases. Approximately 5% of HL belongs to the subgroup of nodular lymphocyte-predominant HL. The tumor cells of cHL are called HRS cells. Hodgkin cells are mononuclear, and Reed-Sternberg cells are bi- or multinucleated variants of the lymphoma clone.

Even though HRS cells most likely originate from germinal center B cells [2–4], they lack expression of most B-lymphocyte markers, including the BCR and transcription factors important for B cell function [5–7]. This “lost B cell phenotype” is an exceptional phenomenon among B cell lymphomas. Moreover, HRS cells express several transcription factors that are normally not expressed by B cells and that are master regulators of other hematopoietic lineages, including inhibitor of DNA binding 2 and NOTCH1 [8–10]. Another characteristic feature of cHL is that the HRS cells usually account for only 1% or a few percent of the cells in the tumor, which is mostly composed of inflammatory cells. The abundance, regular appearance, and heterogeneity of this cellular infiltrate indicate specific roles for these cells in the pathophysiology of cHL. The strict association of HRS cells with their microenvironment and the difficulty to grow HRS cells in culture or in immunodeficient mice indicate a major pathogenetic role of the interaction of HRS cells with the other cells in the microenvironment. It is hence of major relevance to study these interactions and the specific features of the tumor-infiltrating cells.

THE MANY FACETS OF THE CHL MICROENVIRONMENT
The microenvironment in cHL is composed of a large variety of inflammatory and stromal cells, such as several types of T cells, B cells, plasma cells, neutrophils, eosinophils, mast cells, myeloid cells, and fibroblasts. There is substantial variability in the composition of the microenvironment, with few lymphocytes in the lymphocyte-depleted form of HL, numerous B and T cells in lymphocyte-rich cHL, a mixed cellular infiltrate in mixed cellularity HL, and a pronounced occurrence of fibroctic bands in nodular sclerosis HL. Because of the massive infiltration by inflammatory cells, the normal histologic picture of lymph nodes with a separation into B cell follicles and T cell areas is lost. The cellular infiltrate most likely includes cells that aim to eliminate the HRS cells, as well as inflammatory cells that support the survival and proliferation of the tumor clone. There is now evidence that HRS cells actively orchestrate the composition of the lymphoma microenvironment.

CD4+ T cell subsets play a pivotal role in the cHL microenvironment and are attracted by HRS cells that produce large amounts of the chemokines CCL5, CCL17, and CCL22 (Fig. 1) [11–13]. Eosinophils are recruited into the lymphoma through secretion of IL-5, CCL5 [12], CCL28 [14], and GM-CSF [12]. Mast cells and macrophages also may be attracted by CCL5 [15] and neutrophils by IL-8 [12]. Activation and proliferation of fibroblasts, as seen particularly in nodular sclerosis HL, can be

Abbreviations: APCs = antigen presenting cells, APRIL = a proliferation-inducing ligand, BCMA = B cell maturation antigen, CD30/40/95/137L = cluster of differentiation 30/40/95/137 ligand, cHL = classical Hodgkin lymphoma, CTLs = cytotoxic T lymphocytes, DDR2 = discoidin domain receptor tyrosine kinase 2, EBNA1 = EBV nuclear antigen 1, EBV = Epstein-Barr virus, Fasl = Fas ligand, FGF = fibroblast growth factor, GEM-P =

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mediated by HRS cells through secretion of IL-13, TNF-α, and FGF [12]. The activated fibroblasts can then contribute to eosinophil and Th2 cell infiltration by secretion of CCL11 [16].

For a number of cell types attracted by HRS cells into the tumor tissue, there is indication that these cells support the survival and/or proliferation of the HRS cells, as mentioned above. M2 macrophages are induced by MIF, produced by HRS cells [17]. M2 macrophages, for their part, produce MIF and thereby, have supposedly stimulatory effects on HRS cells by the binding of MIF to CD74, which is expressed consistently by HRS cells (Fig. 1) [18]. Several RTKs, expressed on HRS cells, mediate prosurvival effects. Neutrophils produce the NGF that can activate neurotrophic TRKA [19], and HGF = hepatocyte growth factor, neurotrophic TRKA [19], and prosurvival effects. Neutrophils produce the NGF that can activate neurotrophic TRKA [19], and fibroblasts typically produce high quantities of collagen, the main ligand of the RTK DDR2 [20], and the dendritic cell-derived HGF, which activates MET (Fig. 1) [21]. Eosinophils and mast cells are CD30L positive and may contribute to NF-κB activity in HRS cells by stimulating CD30 on HRS cells [22, 23]. Additional factors that stimulate HRS cells are IL-3, which is secreted by CD4+ T cells [24, 25], and APRIL, that is expressed by neutrophils and binds to BCMA and transmembrane activator and calcium-modulating ligand-interactor on HRS cells [26].

Given this complex interplay of HRS cells with the other cells in the HL microenvironment, it is not surprising that HRS cells do not grow as solitary cells in vitro and that only few HRS cell lines could be established that survive, isolated from their cellular niche. The few existing HL cell lines have indeed adopted themselves to the growth in suspension culture without other immune cells [27]. However, as no mouse model for HL exists and as it is not feasible to obtain enough primary HRS cells for in vitro functional studies, HL cell lines are currently the only available model to study functional aspects of HRS cells, including interactions with other cells.

### T CELLS IN THE CHL MICROENVIRONMENT

Typically, the largest population of cells in the cHL microenvironment is T cells [28]. The T cell infiltrate is composed of CD8+ CTLs, CD4+ Th cells, and CD4+ T_{reg}. Initially, the Th cells were regarded as the Th2 type, which is specialized in supporting humoral immune responses [29]. However, a recent study suggests a T cell infiltrate with distinctive Th1 cell features [30]. As Th2, rather than Th1, T cells are generally accepted to favor tumor survival and evasion, a Th1-dominated T cell infiltrate seems contraintuitive. HL, however, is exceptional for its proinflammatory environment. For example, Th1-typical cytokines, such as TNF-α, even secreted by HRS cells themselves, are likely to contribute to tumor survival [31, 32]. Some of the reported discrepancies might be a result of different technical approaches and subpopulations analyzed. Analyses of the cytokine profile neither turn the balance to Th1 or Th2 cells nor could they conclusively characterize the T cell populations in the HL environment [33–35].

The dependency of HRS cells on CD4+ T cells is supported by several findings. These cells always accompany HRS cells, even when HL disseminates into other organs, such as the bone marrow. The dependency of HRS cells on CD4+ T cells may also be one of the reasons why HRS cells, on their own, do not survive in immunodeficient mice or only very rarely in vitro [36]. HRS cells are typically in close contact with and surrounded by CD4+ T cells, a phenomenon that is called rosetting [37]. It is also noteworthy that in patients with the acquired immune-deficiency syndrome, the incidence of HL increased with a beneficial course of disease and concurrent recovery of CD4+ T cells. At the same time, the incidence of other B cell lymphomas dropped, again pointing to a dependency of HRS cells on CD4+ T cells [38].

The rosetting T cells appear to be composed of Th cells and T_{reg} [35, 39]. The intimate contact between the HRS and T cells is partly mediated by several pairs of adhesion molecules, in particular, CD54-CD11a and CD58-CD2 (Fig. 1) [40, 41]. The HRS cell-supportive features of the T cells most likely involve several surface ligand-receptor interactions and soluble factors. CD40 (TNFRSF5) is a member of the TNFRSF and is highly expressed on HRS cells [42, 43]. The triggering of CD40 results in the activation of the NF-κB pathway and enhances colony formation of HRS cells [44, 45]. The aberrant activity of the NF-κB pathway is a hallmark of HL and is additionally facilitated by deleterious mutations in NF-κB inhibitors [46]. It has been shown that, in particular, rosetting T cells express the CD40L, a feature of Th2 cells and T_{reg}, and thereby, may provide survival signals for the HRS cells [43]. Notably, stimulation with CD40L

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induces expression of CD54 on HRS cells [47], which probably contributes to the intimate interaction between HRS cells and the rosetting T cells.

In addition to CD40, CD80 and CD86 (B7-1, B7-2) are further B cell markers with retained expression on HRS cells that are relevant for the cross-talk with T cells [48–50]. These costimulatory B7 antigens bind to CD28 of T cells and putatively contribute to a reciprocal stimulation of HRS cells and surrounding T cells [48]. However, it should be mentioned that HRS cells often have down-regulated MHC-II expression (see also below), so that a fully physiologic cognate interaction between CD4+ T cells and HRS cells appears unlikely. Secretory factors that are secreted by T cells, such as IL-3, also contribute to HRS cell proliferation and survival [24, 25].

Besides the direct, supportive effect of CD4+ T cells on HRS cells, a further, main aspect of the CD4+ and, in particular, Treg between CD4+ T cells and HRS cells appears unlikely. Secretory factors that are secreted by T cells, such as IL-3, also contribute to a reciprocal stimulation of HRS cells and surrounding T cells [48]. However, it should be mentioned that HRS cells often have down-regulated MHC-II expression (see also below), so that a fully physiologic cognate interaction between CD4+ T cells and HRS cells appears unlikely. Secretory factors that are secreted by T cells, such as IL-3, also contribute to HRS cell proliferation and survival [24, 25].

The shear abundance of CD4+ T cells in direct connection with the HRS cells might be beneficial for the tumor cells, simply by shielding them from a direct contact with CD8+ T cells or NK cells. However, there is, most likely, also a major functional role of numerous Treg in the HL microenvironment (Fig. 2) [54, 55]. These cells can inhibit cytotoxic cells by secretion of IL-10, expression of CTLA4, and presumably, several other mechanisms. CTLA4 is also up-regulated on activated Th cells in the HL microenvironment, which may further suppress an anti-HRS cell attack by cytotoxic cells [30, 35, 55].

As HRS cells secrete CCL5, CCL17, CCL20, and CCL22, the tumor cells can attract CCR4-positive Treg into the lymphoma tissue [56]. CD4+CD26+ T cells that were found to be enriched in the HL environment display a distinctive Treg profile [35, 57]. The lack of CD26—a surface protein that proteolytically processes and thereby inactivates chemokines, such as CCL5, CCL11, and CCL22—indicates an attraction of these particular T cells by HRS cells as a result of the specific chemokine secretion [58]. Studies with HL cell lines indicate that HRS cells also have the capacity, e.g., through the secretion of IL-13, to promote the differentiation of naive T cells toward a more Th2 and Treg phenotype [59, 60]. In addition, IL-7 secretion by HRS cells supports the survival and proliferation of Treg [61].

The immunosuppressive activity of Treg is complemented by numerous further strategies of HRS cells to evade an attack by CTL and NK cells. This includes secretion of the immunosuppressive factors TGF-β, MIF, and galectin-1 (LGALS1) [12, 62–66]. LGALS1 was found to be expressed on the surface of HRS cells and secreted by these cells, and its expression correlates with a reduced CD8+ T cell infiltration [63, 64]. Notably, in vitro experiments indicate that LGALS1 secretion promotes a tumor-beneficial shift toward a more Th2 cell-characteristic phenotype [64]. There is also evidence that PGE2 is secreted in the HL microenvironment and contributes to the suppression of T cell activity, but the cellular source of this factor has not yet been identified [67]. HRS cells ectopically express CD137 and transfer this factor by trogocytosis to other HRS and APCs, which causes down-regulation of CD137L in the target cells and as a consequence, reduced T cell stimulation [68].

Differential expression of MHC molecules may also contribute to an immune evasion of HRS cells from CTL. HRS cells

Figure 2. Immune escape mechanisms of HRS cells. Shown are main mechanisms how HRS cells escape from an attack by CTLs and NK cells. The release of CD137 by trogocytosis from HRS cells functions indirectly on the inhibition of T cell activity: CD137 is taken up by HRS and APCs, which causes down-regulation of CD137L in these cells. As a consequence, T cell activation is impaired. MHC-I and -II are down-regulated in HRS cells of a large fraction of cHL cases, which impairs their recognition by CTLs and the activation of Th1 T cells, respectively. Down-regulation of NK group 2, member D, ligands (NK2DL) also impairs activation of CTLs. Gal1, Galectin 1.
frequently down-modulate MHC-I and -II molecules, so that their recognition by CTL or Th cells supporting the cytotoxic activity is presumably dampened. The down-modulation of MHC-I and -II expression in HRS cells is mediated in a fraction of cases by genetic lesions [69–71], supporting an important pathogenetic role of this phenomenon. The nonclassic MHC-I molecule HLA-G is up-regulated on HRS cells. HLA-G is a ligand for inhibitory receptors on tumor-attacking cells [72]. Moreover, the surface expression of FasL (CD95L) and PD-L1 (CD274) and PD-L2 (CD273) also directly shields HRS cells from tumor-specific CTLs or NK cells. Overexpression of FasL, has been described in virtually all HRS cells of nodular sclerosis and mixed cellularity cHL. The expression of CD95L may induce apoptosis of activated, CD95-positive Th1 and CD8+ T cells [73, 74]. The expression of PD-L1 and PD-L2 by HRS cells inhibits T cell activity and can cause T cell exhaustion [62, 75, 76].

Evidence that CD4+ T cells dampen immune surveillance and directly support tumor survival, the identity and features of T cell subsets in the milieu are still surprisingly elusive. In vitro studies to investigate HRS-T cell interactions are hampered by difficulties to obtain sufficient, pure, primary HRS cells. However, eventually overcoming these difficulties might provide further insights for new therapeutic approaches that specifically target tumor-protective T cells.

**AUTHORSHIP**

F.W. and R.K. wrote the review.

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**DISCLOSURES**

The authors declare no conflict of interest.

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