EUROPEAN HEMATOLOGY ASSOCIATION

Chronic lymphocytic leukemia - Section 1

Relevance of the microenvironment in chronic lymphocytic leukemia

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Take-home messages

- Tumor associated macrophages (TAMs) are key cells in the CLL cell microenvironment.
- Extracellular vesicles contribute to the inflammatory and immune-protected tumor-supportive niche.
- It will be important to confirm in human samples the findings obtained in (xenogeneic) mouse models.

Malignant chronic lymphocytic leukemia (CLL) clones are genetic mutineers that develop and thrive within permissive specialized tissue microenvironments provided by secondary lymphoid organs and bone marrow (BM) where the stimulation of the B-cell receptor (BCR) has a pivotal role (*Stevenson FK et a.l, Semin Hematol. 2014*). CLL can be classified into two major subsets that carry unmutated (U-CLL) or mutated (M-CLL) IGHV genes. These CLL subsets are biologically distinct and have different prognosis, but share the common feature of chronic antigen exposure in tissue sites where proliferative events occur (*Stevenson FK et al., Seminars Hematology 2014*) and where leukemic cells entail a bi-directional dialogue with a host of non-malignant elements including cells of both innate and adaptive immunity (*Caligaris-Cappio et a.l, Seminars in Cancer Biology, 2014*). Cell-cell interactions and soluble factors favor malignant cell growth, survival, drug resistance and prevent anti-tumor response. Thereby CLL cell/microenvironment cross talk is a major challenge for effective anti-CLL treatment (**Burger et al., Blood 2009*).

Only a small fraction of CLL cells are able to divide in the so-called "proliferation centers" of primary and secondary lymphoid tissues, where contact with antigen (Ag) and CD4(+)CD40L(+) T cells occurs. The mechanisms leading to CLL proliferation are still uncertain. A significant role appears to be exerted by the subset of T follicular helper (Tfh) cells (*Pascutti MF, et al Blood 2013*) which require BCL-6 and Il-21 (*Jogdand GM et al., Frontiers in Immunology 2016*). A critical aspect of CLL clonal expansion is the incapacity of leukemic cells to differentiate into antibody-producing cells that might be able to neutralize the stimulating Ag. In mouse models the B-cell maturation block appears to be not CLL-cell inherent but microenvironment dependent, a sort of non-classical germinal center–like reaction which takes place in tissue proliferation centers through the activity of Th1-polarized T-bet+ T cells (*Patten et al., JCI Insight 2016*).

As all CLL relevant events occur in tissue microenvironments it is relevant to consider the role of cell traffic and migration, a crawling journey accounted for by the cytoskeleton reorganization of malignant cells. Investigating the incompletely understood mechanisms of malignant cell egress from tissues into peripheral blood (PB) (*Patrussi L et al., Cancer Res 2015*) as well as those allowing tissue entry and re-entry (*Lafouresse F et al., Blood 2015*) starts shedding some light onto the rules that govern the circulation of tumor cells. The core of the problem is which events occur within the involved tissues and how the pro-tumorigenic and immune-protected habitats of CLL microenvironments are established.

Key players in the CLL microenvironment include T cells, stromal cells and nurse-like cells (NLC) (*Figure 1*). A role for monocyte/macrophage cells in CLL had already been suggested by a number of *in vitro* and correlative studies (*Reinart et al., Blood 2013*). As an example NLC are induced upon co-culturing PB or spleen monocytes with CLL cells *(Burger et al., Blood 2000*) and have been identified as CLL-specific tumor-associated macrophages (TAMs) *(Filip et al., Blood Cells Molecules and Diseases, 2013*). Furthermore, the gene expression profile (GEP) of CLL cells exposed *in vitro* to NLCs is remarkably similar to that of CLL cells isolated from lymph nodes *(Herishanu et al., Blood 2011*).

Recently the mechanisms through which TAMs regulate leukemic cell growth *in vivo* have been investigated by means of different CLL transplantation mouse models and TAM depletion strategies (**Galletti et al., Cell Reports 2016; Hanna et al., Leukemia 2016; *Nguyen PH et al., Cancer Cell 2016*). The results demonstrate that TAMs critically support the survival and proliferation of CLL cells *in vivo* and suggest therapeutic strategies based upon manipulating TAM/CLL-

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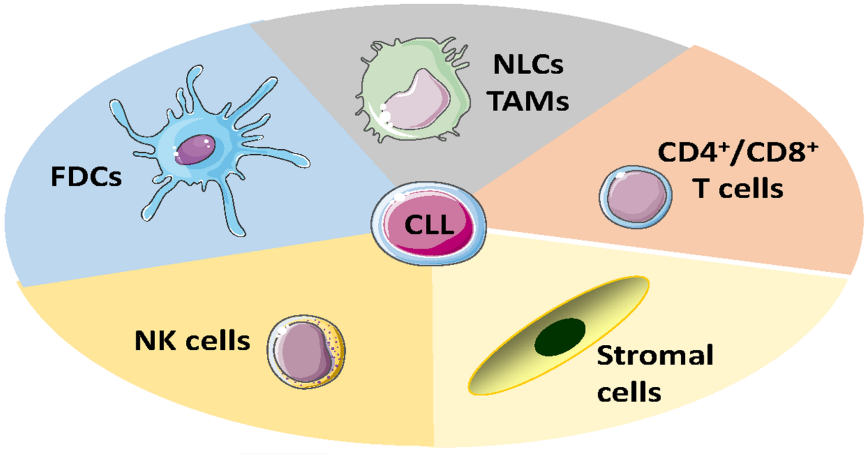
cell interactions (*Galletti et al., Leukemia 2016*). GEP shows that BM monocytes/macrophages exposed to leukemic cells *in vivo* are enriched for specific genes involved in a number of monocyte/macrophage functions (**Galletti et al., Cell Reports 2016*) and that also the transcriptome of leukemic cell is modified underlying the cross talk bi-directionality. The scenario emerging from mouse findings emphasizes that the microenvironment provides critical niches where the engraftment and progression of leukemic clones occur with the help of monocytes/macrophages and that at the same time the leukemic infiltration modifies the function of normal myeloid cells during leukemia development and progression. It will be important to explore whether the information obtained from mouse models can be extrapolated to patients with CLL and to elucidate to what extent this scenario is influenced by the stimulating role of (auto)antigens.

A critical molecule on the surface of monocyte/macrophages is colony-stimulating factor-1 receptor (CSF1R). The therapeutic anti-CSF1R monoclonal antibody (moAb) emactuzumab prevents the formation of new macrophages by inducing apoptosis or inhibiting monocyte differentiation and is an emerging clinical tool to target macrophages *(Ries et al., Cancer Cell 2014; Ruffell and Coussens, Cancer Cell 2015*). In mouse models the anti-CSF1R moAb has been shown to impair CLL cell engraftment and to associate with a striking anti-leukemic effect significantly improving mouse survival (**Galletti et al., Cell Reports 2016*). Mechanistically macrophage targeting sensitizes leukemic cells to apoptosis via induction of TNF signaling pathway and triggers their death through a TNF-dependent mechanism. A critical intracellular signaling molecule has been shown to be Lyn (**Nguyen PH et al., Cancer Cell 2016)*. Evidence has been provided in the Eμ-TCL1 mouse model (**Nguyen PH et al., Cancer Cell 2016)* that, while Lyn deficiency in murine CLL B cells does not influence the malignancy evolution, it supports CLL pathogenesis by operating in the leukemia microenvironment, especially through macrophage function as the loss of Lyn in the macrophages fails to support CLL growth. This mouse finding may be highly significant considering that Lyn had been found to be overexpressed and constitutively activated in human CLL *(Contri et al., J Clin Invest 2005)*.

Within the general context of the interactions between macrophages and leukemic cells it will be relevant to understand if and how the new drugs (e.g., kinase inhibitors) might be influencing *in vivo* macrophages besides exerting their effect on tumor cells.

Key mediators of intercellular communication between CLL cells and the microenvironment are cell-cell contacts through ligand/receptor interactions and exchange of soluble factors. An emerging unanticipated complexity of the mechanisms that account for intercellular communication is exemplified by the cell-cell signalling within the immune system represented by the shedding of extracellular vesicle (EV) (*Robbins and Morelli, Nat Rev Immunol 2014*). EV include exosomes and



FDC: follicular dendritic cells
NLCs: nurse-like cells
TAMs: tumor-associated macrophages

Figure 1. The key cells in the CLL cell neighborhood.

larger micro vesicles: their precise categorization as well as the specific biological significance of the different forms of EV are still problematic. Even with these caveats it has been found that CLL-produced exosomes induce the transition of stromal cells into cancer-associated fibroblasts (CAFs) (*_Paggetti et al., Blood 2015_*) which contribute to an inflammatory tumor-supportive microenvironment creating a niche that promotes CLL development/progression by favoring cell adhesion, survival, and growth. EV may also be involved in shaping an immune-protected niche. The transfer of CLL EV into autologous T cells has been shown to increase T-cell motility, improve the function of immunological synapse and promote the interactions of T cells with leukemic cells (*_Smallwood et al., Blood 2016_*), suggesting that the yet incompletely defined EV molecule cargo might have important immunomodulatory implications.

Taken together these findings shed some light onto which are the key cells in the CLL cell neighborhood and how they communicate. Conceivably the improved understanding of the microenvironment complexity (Figure 1) together with the role of antigen stimulation will soon become clinically meaningful.

References

Stevenson FK, Forconi F, Packham G. The meaning and relevance of B-cell receptor structure and function in chronic lymphocytic leukemia. Semin Hematol 2014;51:158-67.

Caligaris-Cappio F, Bertilaccio MT, Scielzo C. How the microenvironment wires the natural history of chronic lymphocytic leukemia. Semin Cancer Biol. 2014;24:43-8

Burger JA, Tsukada N, Burger M, Zvaifler NJ, Dell'Aquila M, Kipps TJ. Blood-derived nurse-like cells protect chronic lymphocytic leukemia B cells from spontaneous apoptosis through stromal cell-derived factor-1. Blood 2000;96:2655-63.

*Burger JA, Ghia P, Rosenwald A, Caligaris-Cappio F. The microenvironment in mature B-cell malignancies: a target for new treatment strategies. Blood 2009;114:3367-75.

A review outlining how the improved understanding of the microenvironment complexity is becoming clinically meaningful.

Pascutti MF, Jak M, Tromp JM, Derks IA, et al. IL-21 and CD40L signals from autologous T cells can induce antigen-independent proliferation of CLL cells. Blood 2013;122:3010-9. Comments in: Jogdand GM, Mohanty S, Devadas S. Regulators of Tfh Cell Differentiation. Front Immunol 2016;7:520. eCollection 2016.

Patten PE, Ferrer G, Chen SS, et al. Chronic lymphocytic leukemia cells diversify and differentiate in vivo via a nonclassical Th1-dependent, Bcl-6-deficient process. JCI Insight 2016;1:pii: e86288.

Patrussi L, Capitani N, Martini V, et al. Enhanced chemokine receptor recycling and impaired S1P1 expression promote leukemic cell infiltration of lymph nodes in chronic lymphocytic leukemia. Cancer Res 2015;75:4153-63.

Lafouresse F, Bellard E, Laurent C, et al. L-selectin controls trafficking of chronic lymphocytic leukemia cells in lymph node high endothelial venules in vivo. Blood 2015;126:1336-45.

Reinart N, Nguyen PH, Boucas J, et al. Delayed development of chronic lymphocytic leukemia in the absence of macrophage migration inhibitory factor. Blood 2013;121:812-21.

Filip AA, Cise1 B, Koczkodaj D, et al. Circulating microenvironment of CLL: are nurse-like cells related to tumor-associated macrophages? Blood Cells Mol Dis 2013;50:263-70.

Herishanu Y, Pérez-Galán P, Liu D, et al. The lymph node microenvironment promotes B-cell receptor signaling, NF-kappaB activation, and tumor proliferation in chronic lymphocytic leukemia. Blood 2011;117:563-574.

*Galletti G, Scielzo C, Barbaglio F, et al. Targeting macrophages sensitizes chronic lymphocytic leukemia to apoptosis and inhibits disease progression. Cell Rep. 2016;14:1748-60.

This paper demonstrates the critical role of tumor-associated macrophages (TAM) in vivo and suggests novel therapeutic strategies based upon manipulating TAM/CLL-cell interactions.

Hanna BS, McClanahan F, Yazdanparast H, et al. Depletion of CLL-associated patrolling monocytes and macrophages controls disease development and repairs immune dysfunction in vivo. Leukemia 2016;30:570-9.

*Nguyen PH, Fedorchenko O, Rosen N, et al. LYN kinase in the tumor microenvironment is essential for the progression of chronic lymphocytic leukemia. Cancer Cell 2016;30:610-22.

This paper shows that Lyn is a critical signalling molecule that supports CLL pathogenesis by operating in the leukemia microenvironment, especially through macrophage function.

Galletti G, Caligaris-Cappio F, Bertilaccio MT. B cells and macrophages pursue a common path toward the development and progression of chronic lymphocytic leukemia. Leukemia 2016;30:2293-301.

Ries CH, Cannarile MA, Hoves S, et al. Targeting tumor-associated macrophages with anti-CSF-1R antibody reveals a strategy for cancer therapy. Cancer Cell 2014;25:846-59.

Ruffell B, Coussens LM. Macrophages and therapeutic resistance in cancer. Cancer Cell 2015;27:462-72.

Contri A, Brunati AM, Trentin L, et al. Chronic lymphocytic leukemia B cells contain anomalous Lyn tyrosine kinase, a putative contribution to defective apoptosis. J Clin Invest 2005;115:369-78.

Robbins PD, Morelli AE. Regulation of immune responses by extracellular vesicles. Nat Rev Immunol 2014;14:195-208.

*Paggetti J, Haderk F, Seiffert M, et al. Exosomes released by chronic lymphocytic leukemia cells induce the transition of stromal cells into cancer-associated fibroblasts. Blood 2005;126:1106-17.

This paper shows that CLL-produced exosomes contribute to creating an inflammatory tumor-supportive niche that promotes CLL development/progression.

*Smallwood DT, Apollonio B, Willimott S, et al. Extracellular vesicles released by CD40/IL-4-stimulated CLL cells confer altered functional properties to CD4+ T cells. Blood 2016;128:542-52.

This paper shows that extracellular vesicles may have important immunomodulatory functions and be involved in shaping an immune-protected niche in CLL.