

Chronic lymphocytic leukemia cells are active participants in microenvironmental cross-talk

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ABSTRACT

The importance of the tumor microenvironment in chronic lymphocytic leukemia is widely accepted. Nevertheless, the understanding of the complex interplay between the various types of bystander cells and chronic lymphocytic leukemia cells is incomplete. Numerous studies have indicated that bystander cells provide chronic lymphocytic leukemia-supportive functions, but it has also become clear that chronic lymphocytic leukemia cells actively engage in the formation of a supportive tumor microenvironment through several cross-talk mechanisms. In this review, we describe how chronic lymphocytic leukemia cells participate in this interplay by inducing migration and tumor-supportive differentiation of bystander cells. Furthermore, chronic lymphocytic leukemia-mediated alterations in the interactions between bystander cells are discussed. Upon bystander cell interaction, chronic lymphocytic leukemia cells secrete cytokines and chemokines such as migratory factors [chemokine (C-C motif) ligand 22 and chemokine (C-C motif) ligand 2], which result in further recruitment of T cells but also of monocyte-derived cells. Within the tumor microenvironment, chronic lymphocytic leukemia cells induce differentiation towards a tumor-supportive M2 phenotype of monocyte-derived cells and suppress phagocytosis, but also induce increased numbers of supportive regulatory T cells. Like other tumor types, the differentiation of stromal cells towards supportive cancer-associated fibroblasts is critically dependent on chronic lymphocytic leukemia-derived factors such as exosomes and platelet-derived growth factor. Lastly, both chronic lymphocytic leukemia and bystander cells induce a tolerogenic tumor microenvironment; chronic lymphocytic leukemia-secreted cytokines, such as interleukin-10, suppress cytotoxic T-cell functions, while chronic lymphocytic leukemia-associated monocyte-derived cells contribute to suppression of T-cell function by producing the immune checkpoint factor, programmed cell death-ligand 1. Deeper understanding of the active involvement and cross-talk of chronic lymphocytic leukemia cells in shaping the tumor microenvironment may offer novel clues for designing therapeutic strategies.

Introduction

Chronic lymphocytic leukemia (CLL) is a prototypic malignancy that not only depends on intrinsic genetic defects, but is maintained by interactions with bystander cells in microenvironmental niches such as the lymph node. Bystander cells involved include T cells, monocyte-derived cells (MDC), and stromal cells (such as endothelial cells, fibroblastic reticular cells, and pericytes). Signals emanating from these cells critically affect several key features of malignancy of CLL cells,

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such as cell survival, chemo-resistance, cell proliferation, and migration.¹ Moreover, these signals result in an immunotolerant milieu in the CLL lymph node, in which the response to both pathogens² and neo-antigen-expressing malignant cells³ is dampened.

Multiple types of regulators are involved in these communication processes: first, interleukins, such as interleukin (IL)-4 and IL-21, are involved in cell survival and proliferation^{4,5} and IL-10 in immunosuppression.⁶ Second, chemokines, including C-C motif chemokine (CCL)2, 3, 4, and 22, have an important role in chemo-attraction of cells towards the tumor microenvironment (TME).^{7,8} In addition, CCL2 might play a role in tumor cell survival by indirect support via the microenvironment.⁹ Third, growth factors, such as insulin-like growth factor 1, can promote survival.¹⁰ Fourth, membrane-bound factors from bystander cells, such as CD40L and integrins, can induce cell survival.¹¹ Fifth, small vesicles, such as microvesicles and exosomes containing RNA, proteins, lipids or metabolites that are produced by either bystander cells¹² or CLL cells,^{13,14} could transmit signals. Sixth, nucleoside adenosine is involved in dampening the local immune response and causing chemoresistance in CLL cells.¹⁵

Although it is by now well established that the factors secreted by bystander cells are essential for sustaining CLL (summarized in a recent review by Ten Hacken & Burger¹), it has also become clear that these interactions are reciprocal in nature. As shown in other tumor types, upon contact with tumor cells, bystander cells can undergo changes that drive tumor progression.⁷ Considering that CLL bystander cells include immune cells normally involved in highly adaptable immune responses, they are highly susceptible to (malignant) B-cell-derived signals. Alongside local changes leading to tumor progression, bystander cell alterations lead to systemic changes that can orchestrate recruitment of peripheral cells towards the TME.⁷ Although various studies have suggested that bystander cell changes can take place at the genetic level,⁷ recent evidence has shown unaltered stromal genomes, suggesting that microenvironmental signals are not mediated via genetic events.⁷ These findings indicate that the stromal alterations are reversible, and that identification of the factors driving stromal cell changes may yield new therapeutic options.

In this review we analyze contemporary literature and our own recent findings to provide an overview of current evidence that signals emanating from CLL cells are crucial in creating a tumor-supportive TME. Second, as several reports show interdependency of bystander cells, we address how communication among bystander cells can contribute, in the context of CLL, to supportive TME interactions. We focus on T cells, MDC and stromal cells which together with CLL cells can form a tetrad exchanging reciprocal signals. For each of these, the functional effects of CLL cells towards the bystander cells are discussed followed by the relevant mechanisms. Lastly, we discuss effects between bystander cells.

T-cell interactions

Although it has been described that CD4⁺ T helper type 1 (Th1) cells recognize CLL antigens,³ activated Th1 cells also induce CLL-cell proliferation and survival.¹⁶ Furthermore, T cells activate mitochondrial metabolism in CLL cells, which renders CLL cells more resistant to chemotherapy and contributes to cell proliferation.¹⁷ Pro-

tumor signals from T cells include both antigen-independent proliferation factors (CD40L in combination with IL-21⁵) as well as survival inducing factors [interferon (IFN)- γ ,¹⁸ IL-4,⁴ and CD40L¹⁹] (Figure 1). These pro-survival signals result in a nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B)-dependent upregulation of B-cell lymphoma 2 (BCL2) family members BCL2-related protein A1 (BFL-1) and B-cell lymphoma-extra large (BCL-X_L),²⁰ and protein kinase B (AKT)-dependent upregulation of induced myeloid leukemia cell differentiation protein (MCL-1).²¹ With respect to CLL proliferation, mitogen-activated protein kinase (MAPK) and signal transducer and activator of transcription (STAT)3 pathways play additional roles.²²

The interaction of CLL cells with T cells sensitizes CLL cells to additional TME-derived pro-tumor signals; first, B-cell receptor (BCR) signaling is enhanced by a microRNA-155-dependent mechanism after CD40L stimulation.²³ Second, CLL cells upregulate adhesion protein CD44 after CD40L stimulation, leading to hyaluronic acid binding, which increases retention in the lymph node.²⁴ Third, alongside a direct survival-inducing effect of T-cell-secreted IFN- γ on CLL cells, CD38 is upregulated on CLL cells after IFN- γ stimulation. CD38 can subsequently relay MDC-derived CD31 survival signals,²⁵ although this has been difficult to confirm *in vitro*.²⁶ These findings indicate that the pro-tumor effects of T cells might be partially mediated via other TME elements.

Various groups have described aberrations in the T-cell population in CLL patients. The total number of both CD4⁺ and CD8⁺ T cells is increased²⁷ and a skewing of their ratio towards CD8⁺ cells occurs in both mice²⁸ and humans.²⁹ This skewing does not precede the occurrence of CLL, as it is not present during monoclonal B-cell lymphocytosis,³⁰ but even at an early stage of CLL, expansion of the CD8⁺ T-cell population is correlated with an adverse outcome.²⁹ These findings indicate that CLL cells are the causative agent in this correlation. Furthermore, with respect to T-cell developmental stages, an increase in effector cells at the expense of naïve cells is observed.³¹ The functional consequences of this skewing are currently unknown, but it could be speculated that a decreased naïve T-cell pool reduces the number of potential cytotoxic T cells directed towards CLL neo-antigens. Alongside the effects of CLL cells on T-cell skewing, CLL cells induce an exhausted T-cell phenotype.³² This phenotype is characterized by increased expression of exhaustion markers CD160, CD244, BLIMP-1, and programmed cell death protein (PD)-1³² and an inability to produce adequate levels of immune-activating cytokines upon stimulation,³³ similar to the phenotype of T cells directed towards chronic virus infections. Concurrently, effective synapse formation of T cells is suppressed by causing non-polarized release of lytic granules.³⁴ These mechanisms likely contribute to T-cell dysfunction. Lastly, CLL cells are involved in the induction of migration of T cells towards the lymph node.⁸

Several mechanisms have been linked to the observed suppression of T-cell function by CLL cells; it has been observed that CLL cells overexpress immune inhibitory factors such as programmed death ligand (PD-L)1 and PD-L2³⁵ and T cells from CLL patients have increased levels of the PD-1 receptor.²⁹ As PD-1 expression also increases with age, these observations should be interpreted with caution. To study causality, the E μ -TCL1 mouse model, in

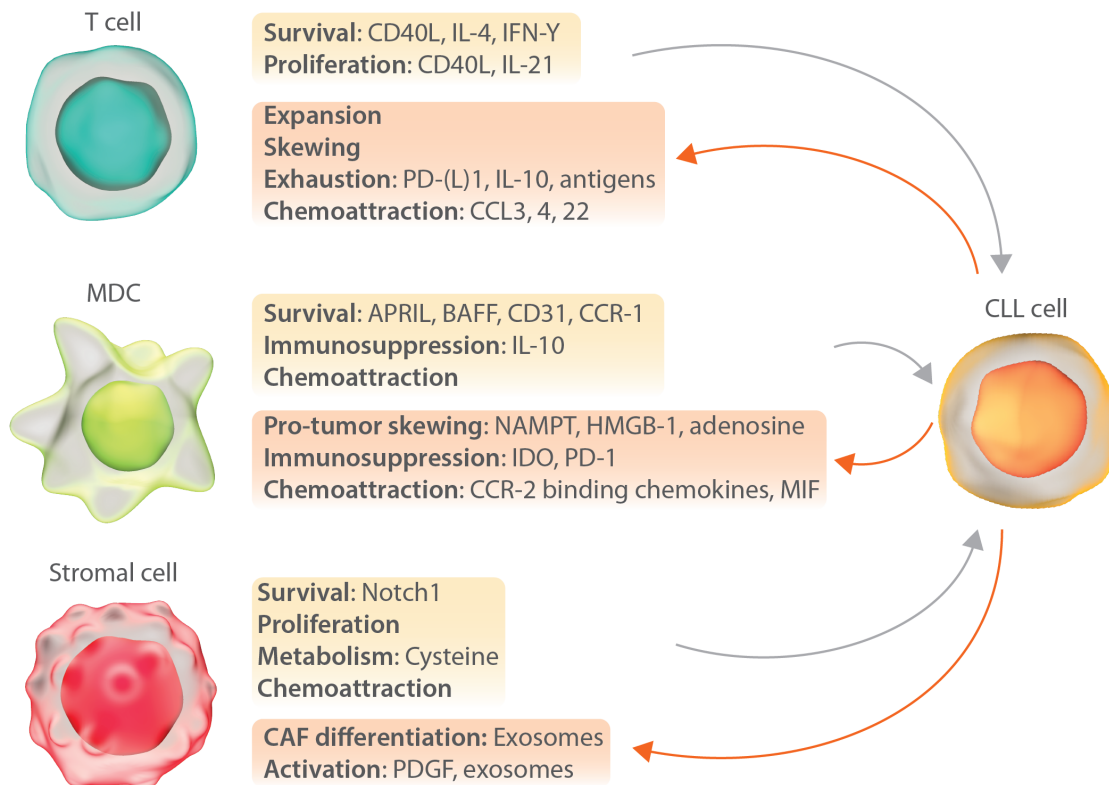


Figure 1. Interactions between chronic lymphocytic leukemia cells and bystander cells that contribute to the formation of a tumor-supportive microenvironment. Within the tetrad of CLL cells, T cells, MDC, and stromal cell, relevant effects (in bold) and signaling molecules involved in the interaction of CLL cells with T cells, MDC, and stromal cells are indicated. IL: interleukin; CCL: chemokine (C-C motif) ligand; IFN: interferon; APRIL: a proliferation inducing ligand; BAFF: B-cell activating factor; NAMPT: nicotinamide phosphoribosyltransferase; HMGB-1: high mobility group box 1; IDO: indoleamine 2,3-dioxygenase; MIF: migration inhibitory factor; CAF: cancer associated fibroblasts; PDGF: platelet derived growth factor.

which oncogene T-cell leukemia/lymphoma protein 1 (TCL1) is overexpressed under control of the B-cell-specific immunoglobulin heavy enhancer, has been used.³⁶ Although CLL in this mouse model is mainly driven by TCL1 in contrast to heterogeneous drivers in human disease, findings in this model have been valuable in explaining at least some of the observed immune disturbances in human CLL.³⁶ Using this model, aging bias was excluded by showing that adoptive transfer of CD19⁺ cells of either wild-type or TCL-1 donor mice towards young wild-type recipients also induces PD-1 on T cells.³⁵ Alongside PD-1-mediated signaling, CLL cells produce the immune inhibitory cytokine IL-10.⁶ Also, unknown contact-dependent factors produced by CLL cells actively impair T-cell synapse formation.³⁷ In addition, adenosine, which is produced in the hypoxic CLL TME, can also contribute to decreased T-cell proliferation.¹⁵

Very recently, a link between CLL-mediated T-cell dysfunction and altered immune metabolism was made by showing CLL-mediated suppression of T-cell glucose metabolism.³⁸ Whether impaired metabolism is a direct consequence of competition for fuels between the tumor cells and T cells, as has been shown in experimental models in other tumors,³⁹ or is solely due to CLL-mediated decreased AKT/mTOR signaling³⁸ has still to be resolved. It is important to note that the mechanistic causes of T-cell

expansion and skewing remain largely obscure, but the defects in T-cell function might underlie the compensatory expansion seen in CLL patients.²⁹

Several factors secreted by CLL cells can induce migration of T cells towards the CLL lymph node. CCL22 for instance, is secreted by CLL cells in the lymph node, which results in the recruitment of T cells.⁹ Interestingly, as CCL22 preferentially induces migration of T helper type 2 (Th2) and T regulatory (T_{reg}) CD4⁺ cells,⁴⁰ secretion of this chemokine could lead to skewing in the lymph node towards CLL-supporting and immunosuppressive T cells at the expense of cytotoxic T cells. Together with T-cell recruitment via CCL22, CLL cells secrete CCL3 and CCL4 upon interaction with MDC⁴¹ and levels of CCL3 correlate with increased T-cell numbers in CLL lymph nodes.⁴² Finally, the fact that T cells show reduced motility upon direct contact with CLL cells⁴³ could indicate that T cells are retained in the lymph node once recruited.

It has recently become evident that CLL cells also affect the phenotype of non-conventional T cells. A small population (1-10%) of the total T-cell pool carries the highly conserved $\gamma\delta$ T-cell receptor (TCR) instead of the more prevalent $\alpha\beta$ TCR.⁴⁴ Within this population, V γ 9V δ 2 T cells are the predominant subset present in the peripheral blood. In contrast to the recognition of peptide antigens by $\alpha\beta$ T cells, V γ 9V δ 2 T cells respond to stress molecules

in malignant cells, in a TCR-dependent yet major histocompatibility complex (MHC)-independent process. As a consequence, these $\gamma\delta$ T cells could suppress CLL cells acting independently of MHC antigen presentation.⁴⁴ Compared to cells from healthy donors, however, these $\gamma\delta$ cells show a dysfunctional phenotype in CLL.⁴⁵ Interestingly, we found that these defects are spontaneously reverted when patient-derived $\gamma\delta$ T cells are cultured in the absence of CLL cells,⁴⁶ in support of continuous, active subversion by CLL cells.

In their role as immunosuppressive cells, T_{reg} cells, on the other hand, secrete several immunosuppressive cytokines such as IL-10 and their number correlates with worse prognosis in several tumors.⁴⁷ In CLL, the frequency of forkhead box protein (FOXP3)⁺ T_{reg} cells is increased in advanced disease.⁴⁸ IL-10 production by T_{reg} cells is higher in the CLL lymph node than in peripheral blood,⁴⁹ in accordance with microenvironmental signals engaging in immunosuppressive skewing.

Monocyte-derived cell interactions

MDC include monocytes, macrophages, and dendritic cells. These cells can, on the one hand, secrete essential survival factors for CLL cells, while, on the other hand, they can potentially mount an immune response against malignant cells as co-stimulators of B- or T-cell-mediated responses.⁵⁰ According to the dichotomized view of macrophage differentiation proposed in normal biology, M1 differentiated immunogenic macrophages mainly convey anti-tumor signals, while M2 wound healing macrophages are pro-tumorigenic overall.⁵¹ The delayed disease development associated with MDC depletion in the TCL1 mouse model^{52,53} suggests that MDC have a crucial, tumor-supportive function in CLL. Their supportive role is further indicated by the observation that a higher number of MDC correlates with worse prognosis in CLL patients.⁵⁴ Whereas MDC play important roles in inducing CLL-cell survival⁵⁵ and have migratory effects on CLL cells⁵⁵ (Figure 1), their role in inducing proliferation is subordinate; stimulation of CLL cells by macrophages does not induce proliferation (*unpublished observation*) and furthermore no spatial correlation between the MDC marker CD68 and the proliferation marker Ki67 exists in lymph nodes from CLL patients.⁵⁶ We recently found that MDC-mediated survival depended on chemokine signaling via CCR1.²¹ Nurse-like cells are monocyte-derived cells which develop following prolonged *in vitro* culture with CLL cells⁵⁵ and have been identified in both the spleen and lymph nodes of CLL patients.⁵⁷ Nurse-like cells are thought to induce CLL survival effects via factors such as A proliferation inducing ligand (APRIL), B-cell activating factor (BAFF) or C-X-C motif chemokine (CXCL)12 (reviewed by Ten Hacken & Burger¹). In line with this, transgenic APRIL overexpression in the TCL1 mouse led to faster disease progression.⁵⁸ By contrast, using a novel APRIL-overexpression system and an APRIL decoy receptor, we recently found *in vitro* that direct effects of APRIL produced by macrophages on CLL cells are negligible.⁵⁶ This discrepancy could be reconciled by postulating that *in vivo* effects of APRIL may be indirect, as exemplified by the recent finding that immunosuppressive IL-10 is produced by non-malignant B cells upon stimulation of APRIL receptor TACI.^{59,61}

In line with the overall pro-tumor effect of CLL-associated MDC, pro-tumor M2 differentiation of macrophages

in the presence of CLL cells has been found *ex vivo* and *in vitro*.⁶²⁻⁶⁴ Functionally, these cells show impaired immunocompetence, as antigen presentation and immune response initiation are decreased.⁶⁵ In addition, CLL-associated monocytes are defective in their phagocytic function.⁶⁶ Moreover, dendritic cells in mice that have undergone adoptive transfer of TCL1 CLL cells show a decrease of MHC class II expression and an increase of the immunosuppressive molecule PD-L1,⁵² and in CLL patients they have a suppressed immature phenotype showing decreased proliferation and cytokine production after stimulation.⁶⁷

Several groups,^{53,68} including our group,⁵⁶ have found that the CLL lymph node is interspersed with macrophages. As recruitment of these supportive macrophages depends on chemokine gradients emanating from the lymph node, it is postulated that CLL cells can provide these migratory signals. Indeed, it has recently been shown that in the TCL1 mouse model, CLL-infiltrated tissues harbor an increased number of monocytes compared to non-transgenic mice.⁵²

Several CLL-secreted factors have been suggested to contribute to the pro-tumorigenic M2 differentiation of monocytes, which include nicotinamide phosphoribosyltransferase (NAMPT)⁶⁵ and high mobility group box 1 (HMGB1).⁶⁸ As NAMPT is also secreted by CLL-differentiated MDC, it could form a positive feedback loop keeping MDC in a CLL-supportive state.⁶⁵ Furthermore, by generating a hypoxic TME, CLL cells indirectly induce M2 differentiation as hypoxia increases adenosine production by MDC, which is known for its M2 differentiating capacity.¹⁵ Besides the direct effects of these factors in inducing M2 differentiation, CLL-associated monocytes are primed for M2 differentiation as they show increased phosphorylation of downstream STAT molecules in response to M2-differentiating cytokines IL-4 and IL-10.⁵² The persistent M2-differentiating signals emanating from CLL cells residing in the lymph node, in combination with PD-1 stimulation,⁶⁹ could explain the immune dysfunction of MDC. These effects are further enhanced by autocrine stimulation via PD-L1 or IL-10 expressed by the MDC themselves.⁵² Furthermore, IL-10 is responsible for immune dysfunction seen in dendritic cells,⁶⁷ as here it leads to STAT6 suppression via suppressor of cytokine signaling 5.⁷⁰

Interestingly, the tumor supportive phenotype in MDC is reversible, as IFN- γ stimulation results in transdifferentiation of pro-tumorigenic (M2) CLL-associated monocytes towards M1 macrophages.⁷¹ Similarly, inhibiting PD1 signals could restore macrophage function,⁶⁹ suggesting there is potential for therapeutic intervention in these pathways.

Although several chemokines could account for the recruitment of monocytes towards the CLL lymph node, a critical role for CCR2 has recently been proposed. Adoptive transfer of CLL cells from TCL1 mice to CCR2 knockout mice led to a decrease in monocyte numbers in the spleen.⁵² We recently found that primary CLL cells are able to secrete several monocyte-attracting chemokines such as CCL2, 3, 4, 5, 7, and 24, and CXCL5 and 10 after stimulation with the T-cell factor CD40L, resulting in monocyte migration. In line with data from Hanna *et al.*,⁵² specific inhibition of CCR2 by small molecules could completely abrogate the migration towards CLL cells.⁶² Others have found that knockout of macrophage migra-

tion inhibitory factor (MIF) reduced the number of macrophages in the spleen of TCL1 mice, suggesting an additional role for this chemokine in MDC migration.⁷²

Stromal-cell interactions

Stromal cells constitute the connective tissue of organs and supply them with structure, anchoring and supportive signals. By definition, they are of non-hematopoietic origin. Different types of stromal cells include fibroblasts, reticular cells, and endothelial cells. Stromal cells can play a supportive role in various tumor environments, including the CLL lymph node⁷³ and stromal cell numbers generally correlate with tumor progression and worse prognosis.⁷⁴ Via several mechanisms, stromal cells can directly support CLL cells, for example, by inducing chemoresistance, promoting migration, and increasing cell survival via factors such as NOTCH1 (reviewed by Ten Hacken & Burger¹). In addition, they induce CLL-cell proliferation⁷⁵ and change CLL-cell metabolism⁷⁶ (Figure 1). At the level of metabolism, stromal cells can supplement the defective CLL cystine transport by secreting large amounts of cysteine into the TME.⁷⁷

Alongside these direct effects, stromal cells can govern changes in CLL cells that make them more receptive to other microenvironmental signals. Upon co-culture with stromal cells, CLL cells upregulate transcription factor hypoxia-inducible factor-1 α , which can induce changes in chemokine receptor expression in CLL cells that consequently retain them in the TME.⁷⁸

As is the case for T cells and MDC, the stromal-cell secretome depends on the extracellular signals it receives. In order to provide tumor support, different stromal cell types have been shown to transdifferentiate into so-called cancer-associated fibroblasts in different malignancies.⁷⁴ In CLL, it has been suggested that this differentiation takes place via specific microRNA delivered through exosomes.¹⁴ To support CLL cells, cancer-associated fibroblasts require AKT signaling.⁷⁹ A bidirectional cross-talk in which CLL cells induce AKT and extracellular signal-regulated kinase (ERK) signaling has been described⁸⁰ and platelet-derived growth factor is one secreted factor that can cause this activation.⁸¹ In summary, these mechanisms underpin the dependence on CLL-secreted factors for tumor-supportive differentiation of stromal cells.

Interactions between bystander cells: monocyte-derived cells and T cells

We have so far discussed several direct reciprocal interactions between CLL cells and bystander cells. Considering that all cells within an ecosystem partake in reciprocal signaling, interactions between bystander cells can likewise contribute to the formation of a supportive TME in CLL.

Based on their role in the normal immune response, it is to be expected that MDC can also affect the phenotype of T cells in the context of CLL. Indeed, MDC contribute to T-cell skewing in CLL as skewing was reverted after depletion of MDC via clodronate treatment in the TCL1 mouse model.⁵²

Furthermore, MDC are involved at several levels of T-cell suppression; first, in the context of CLL, MDC induce expression of PD-1 on T cells,⁶³ while PD-L1 is upregulated on CLL-differentiated monocytes,⁵² both contributing to T-cell suppression. Second, CLL-differentiated monocytes inhibit T-cell proliferation⁶³ and third, they can inhibit T-

cell activation and promote the differentiation towards T_{reg} cells via secretion of immunosuppressive indoleamine 2,3-dioxygenase (IDO).⁶⁴

Like CLL cells, CLL-differentiated MDC can secrete chemokines that can attract T cells towards the lymph node, such as CXCL12. Furthermore, this chemokine enhances the expression of CLL-cell survival stimuli such as IFN- γ by T cells.⁸² Similarly, in mouse studies, splenic monocytes show increased levels of T-cell-attracting chemokines such as CXCL9 and 10 after adoptive transfer of TCL1 CLL cells.⁵² Concurrently, expression of the receptor for these chemokines (CXCR3), increases on T cells.⁵² This indicates that supporting cells are not only recruited to the TME via induction of attracting chemokines in the lymph node, but also by an increased susceptibility to recruitment via chemokine receptor upregulation.

A subset of MDC, myeloid-derived suppressor cells (MDSC; expressing CD11b and CD33 and low levels of human leukocyte antigen-DR), provides important tumor-supportive factors in several other malignancies due to its immunosuppressive nature.⁸³ In CLL, it has been shown that the MDSC population is expanded^{64,84} and that T cells are suppressed by MDSC.⁶⁴ Furthermore, the number of MDSC correlated with the number of CLL cells in patients.⁸⁴ These data indicate that MDSC might also suppress the T-cell response in the context of CLL.

Conclusion and therapeutic consequences

Cellular cross-talk is the driving force in establishing supportive interactions between elements within the TME. In this review, we have described several CLL-supportive mechanisms by bystander cells and the contribution of CLL cells. It is, however, important to keep in mind that CLL is an intra- and inter-tumoral genetically heterogeneous disease and that several of the described supportive TME mechanisms might depend on a specific genetic background. As an example, CLL cells harboring a NOTCH1 mutation might be more sensitive to NOTCH1 ligands present in the TME.⁸⁵ Likewise, a subset of CLL, specifically cases that harbor the trisomy 12 aberration, overexpresses CD49d, which might make them more sensitive to lymph node homing.⁸⁶ In the same vein, it has been shown that CD38-overexpressing CLL cells are more reactive to (microenvironmental) CXCL12 signaling and BCR signals.⁸⁷ Lastly, *IGHV* mutation status and expression levels and mutations of intracellular BCR signaling proteins such as zeta-chain-associated protein kinase 70, spleen tyrosine kinase, and Bruton tyrosine kinase (BTK) can dictate CLL-cell responses to TME BCR signals.¹ This shows that the receptiveness of CLL cells to TME support and subsequent disease outcome might depend on genetic alterations specific to CLL patients or to particular clones.

With these caveats in mind, we here discuss potential consequences for CLL therapy. With the advent of new treatment modalities for CLL, the potential side-effects that novel therapies have on bystander cells should be considered. For instance, because MDC-mediated antibody responses depend on BTK,⁸⁸ ibrutinib treatment reduces Fc γ R-mediated cytokine production,⁸⁸ inhibits activation,⁶⁹ and changes metabolism⁶⁹ in monocytes, which can inhibit their immune function, as has been shown for antibody-dependent cell-mediated cytotoxicity.⁶⁹ The outgrowth of adoptively transferred CLL cells

was, however, impaired in Btk knockout recipient mice, and macrophages deficient for its upstream tyrosine-protein kinase, Lyn, showed diminished CLL-supportive capacity *ex vivo*.⁹⁰ This suggests that the effects of ibrutinib on macrophages would be clinically beneficial. The depletion of immunosuppressive MDSC by ibrutinib⁹¹ could furthermore support its beneficial clinical effects. Lastly, ibrutinib targets the T-cell-expressed BTK homolog, interleukin-2-inducible kinase (ITK), which is an important modulator of T-cell signaling and function.⁹² Interestingly, as ITK inhibition preferentially affects Th2 cells because Th1 cells express a compensatory kinase, a potentially beneficial Th1 anti-tumor skewing occurs,⁹² as was recently observed *in vivo* in pancreas carcinoma-engrafted mice.⁹³ In the context of chimeric antigen receptor T-cell therapy, T-cell expansion and increased tumor clearance were found when this therapy was used concurrently with ibrutinib,⁹⁴ indicating that ibrutinib treatment can overcome the suppressive effects of CLL cells on T cells. The effects of the kinase inhibitor idelalisib on bystander cells are generally CLL-supportive, as idelalisib reduces cytotoxic cytokine production of T cells⁹⁵ and in macrophages it reduces antibody-dependent cell-mediated cytotoxicity⁹⁹ and migration,⁹⁶ although specific inhibi-

tion of phosphoinositide 3-kinase γ leads to an immunostimulatory macrophage differentiation.⁹⁷ Given the critical pro-tumor effects of bystander cells, these findings suggest that complete tumor eradication after debulking treatment with chemotherapeutics can only be achieved after restoration of T-cell function by ibrutinib⁹⁴ or lenalidomide,⁹⁸ which can be complemented with CLL-directed chimeric antigen receptor T cells and PD-L1 inhibition.⁹⁹ In addition, as ibrutinib treatment results in migration of CLL cells out of the lymph node, subsequent CLL-attracting chemokine inhibition could avoid (re)formation of a tumor-supportive microenvironment and increase the effectiveness of cytotoxic therapies. The effectiveness of this strategy of migration inhibition has been shown, for instance, *in vivo* in prostate cancer, in which metastases were reduced after CXCR4 inhibition.¹⁰⁰ In conclusion, future insights into the dynamics of cellular interactions and the effects of (existing) therapies on these dynamics would substantially aid in designing optimal treatment strategies.

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