

The strange case of nurse cells: Dr. Jekyll or Mr. Hyde?

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Abstract

In developmental biology and physiology of reproduction the term nurse cells refers to cells which provide feeding, support, and stability to their neighboring cells. The notion of nurse cell is used in several unrelated ways in various scientific fields, such as parasitology, mycology, invertebrate and vertebrate development. In this review, we focus on “nurse cells” described to support hemopoietic cell differentiation which can act as a double-edged sword in normal versus pathological conditions. Particularly, the nurturing capacity of stromal cells to assist the differentiation and survival of normal cells within both the bone marrow or the synovium can turn to detrimental effects. In the later cases, nurse cells induce and maintain inflammation and rheumatological disease. Similarly, the anti-tumoral potential of myeloid cells might be switched to tumor promoting function in most cancers and leukemia, including chronic lymphocytic leukemia. A better understanding of the interactions between pathological cells and supporting cells could inspire new drug combination strategies, translational studies, and novel therapeutic options in clinical trials.

Keywords: Nurse cells, Tumor microenvironment, Inflammation, Homeostasis, Chronic lymphocytic leukemia, Rheumatoid arthritis

Abbreviations: $\alpha\beta$ TCR: alpha-beta T Cell Receptor; APRIL: A Proliferation-Inducing Ligand; α SMA: alpha-smooth muscle actin; BAFF: B-cell Activating Factor; BCL2: B-cell Lymphoma 2; Bcl-xL: B-cell lymphoma-extra large; BDNF: Brain-Derived Neurotrophic Factor; BCR: B-cell Receptor; BM: Bone Marrow; BMSC: Bone Marrow Stromal Cell; BM-MS: Bone Marrow Mesenchymal Stem Cell; BTK: Bruton's Tyrosine Kinase; CSF: Colony Stimulating Factor; CLL: Chronic Lymphocytic Leukemia; FDC: Follicular Dendritic Cell; FLS: Fibroblast-Like Synoviocytes; FRC: Fibroblastic Reticular Cells; GM-CSF: Granulocyte-Macrophage Colony-Stimulating Factor; HMGB1: High Mobility Group Box 1; HSC: Hematopoietic Stem Cell; HSNC: Human Skin Nurse Cells; LN: Lymph Node; MAPK: Mitogen-Activated Protein Kinase; MDSC: Myeloid-Derived Suppressor Cell; MSC: Mesenchymal Stromal/Stem Cell; MHC: Major Histocompatibility Complex; NLC: Nurse-Like Cell; OA: Osteoarthritis; PD-L1: Programmed Death-Ligand 1; RA: Rheumatoid Arthritis; RA-NLC: Rheumatoid Arthritis Nurse-like Cell; SDF-1: Stromal Cell Derived factor 1; TADC: Tumor Associated Dendritic Cell; TAM: Tumor Associated Macrophage; TAN: Tumor Associated Neutrophil; TEM: Trans-Endothelial cell Migration; TGF- β 1: Transforming Growth Factor beta; TME: Tumor Microenvironment; TNC: Thymic Nurse Cell; TNF Tumor Necrosis Factor; VCAM-1: Vascular Cell-Adhesion Molecule-1; WBC: White Blood Count

Introduction

A nurse cell is generally defined as a cell specialized in direct interaction with other cells and assisting them in their development by delivering different factors and stimuli and by providing stability. So far, in different tissue contexts, several “nurse cells” with different origins have been described, according to their nurturing capacities. The most intriguing feature of these cells is their dual and opposite behavior as Dr. Jekyll and Mr. Hyde [1]. The “good” behavior is their support to the development and survival of healthy cells as well as the elimination of aberrant cells in frame with physiological events such as homeostasis and immune resolution. The “evil” one covers their roles in exacerbating inflammation and tissue damage and in maintaining tumoral cells allowing immune escape.

Thymic nurse cells (TNC)

The best known nurse cells are thymic epithelial cells (TNC) that temporally interact with and internalize T lymphocyte progenitors in specialized vesicles called caveoles [2] to sustain their

development, maturation and selection. Particularly, the interaction between TNCs and immature T lymphocytes is necessary for their viability during stages of intrathymic development [3]. The active migration of thymocytes into the cytoplasm of thymic nurse cells is called “emperipolesis” [4]. Thymocytes are then trapped in unique membrane structures which provide a microenvironment for a functional interaction between the $\alpha\beta$ TCR on thymocytes and MHC molecules expressed on these vacuoles. Low affinity interactions between the $\alpha\beta$ TCR and MHC molecules allow single-positive thymocytes to be released from the thymus as functional T cells. On the other hand, thymocytes producing an $\alpha\beta$ TCR that binds tightly to self-peptide in association with MHC antigens are not allowed to mature since they are potentially autoreactive if released from the thymus. Similarly, thymocytes producing nonreactive $\alpha\beta$ TCRs are deleted by neglect. By participating in the positive and negative selection of immature T lymphocytes to tolerate self-antigens and recognize foreign antigens, TNCs support the development and survival of healthy T cells and enhance the elimination of aberrant or damaged T cells [3,5,6] (Figure 1).

Nurse cells might also play important roles in extrathymic lymphocytes activation. Nurse-like clones could be established from human skin (HSNCs) and these clones were considered of

mesenchymal origin according to their morphology and markers expression. Interestingly, HSNC induced cytotoxicity against altered autologous cells suggesting that they can act as well on T cell proliferation or selection in the skin, as thymic nurse cells do in the thymus [7]. Particularly, HSNC may impact migration, proliferation and/or differentiation of lymphocytes during inflammatory process in the skin, thus playing a key role in self-defense mechanism against external aggression

Indeed, these findings argue for the presence of nurse-like cells, with functions similar to thymic nurse cells, in other tissue compartments.

In this context, several key players of immune homeostasis could be considered as “beneficial nurse cells” considering their nurturing and patrolling functions.

The “Good” or Beneficial Side

Fibroblast-like synoviocytes and mesenchymal stromal/stem cells in joints homeostasis

The synovium assures joint homeostasis not only through the secretion of critical factors for the joint lubrication but also by supporting repair and differentiation. The main synovium cellular

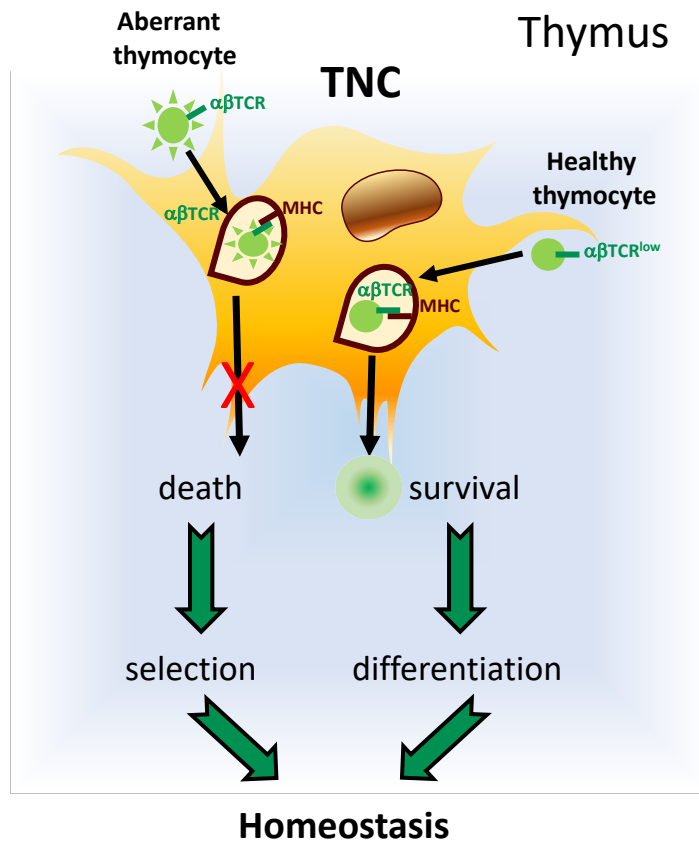


Figure 1. Proposed mechanism of thymic nurse cell role in T cell intrathymic development. Immature thymocytes migrate into the cytoplasm of thymic nurse cells (emperipolesis) and are trapped in specialized caveoles. Low affinity interactions between the $\alpha\beta$ TCR on single-positive thymocytes (healthy thymocytes) and MHC molecules expressed on these vacuoles allow thymocytes to exit the thymus as functional T cells. Thymocytes producing an $\alpha\beta$ TCR that binds tightly to self-peptide in association with MHC antigens or an unreactive $\alpha\beta$ TCR are eliminated. TNC: Thymic Nurse Cell.

components are fibroblast-like synoviocytes (FLS), macrophage-like synovial cells and mesenchymal stromal/stem cells (MSC). FLS produce extracellular matrix (ECM) components, direct matrix assembly and control ECM turnover to influence the synovium matrix structure [8]. Synovial macrophages are involved in immune defense, while mesenchymal stem cells (MSC) proliferate *in vivo* in response to injury and differentiate *in vitro* into chondrocytes, osteocytes, myocytes or adipocytes [9]. MSCs modulate local inflammation and fibrosis, and interact with different fibroblast subtypes and with resident macrophages. In this regard, several clinical trials with arthritic patients demonstrated that BM-MSCs dampen the low-grade synovial inflammation associated with osteoarthritis [10]. MSCs form a heterogeneous population mostly of synovial origin but can also derive from pericytes, endothelial cells, adipose tissue, ligaments, menisci, and from a small subset of bone marrow MSCs (BM-MSCs), which could migrate more directly from subchondral BM into the adjacent synovium. In BM, BM-MSCs are capable of differentiating into various cell types (mainly bone, fat, and cartilage) and provide a supportive microenvironment for the maintenance of hematopoietic stem cells (HSCs) and for hematopoiesis. Furthermore, they also exert modulatory effects on cells of both the innate and adaptive immune responses. Notably, CXCL12-positive BM-MSCs regulate immunological memory by organizing survival niches for plasma cells, while CXCL12-negative BM-MSCs sustain memory T cells by maintaining T cells quiescent through IL-7 expression. They can also induce the differentiation of regulatory T cells (Tregs) and maintain their inhibitory functions, as well as the Tregs/ T helpers balance [11].

Stromal and myeloid cells during hematopoiesis and immune homeostasis

Hematopoiesis takes place in a specialized BM microenvironment, the so-called “niche”, which provides essential autocrine, endocrine, and paracrine signals as well as direct cell-to-cell interactions necessary for the self-renewal and the differentiation into all blood-cell lineages of HSCs [12]. In a similar manner, secondary lymphoid organs (SLOs), including lymph nodes (LNs), spleen and Peyer’s patches (PPs), provide the infrastructure to maintain immune homeostasis and to facilitate rapid and effective immune responses. The characteristic hallmark of SLOs is the highly ordered compartmentalization of T cells, B-cells and myeloid cells to generate optimal antigen-specific adaptive immune responses. Non-hematopoietic lymph node stromal cells (LNSCs), consisting of both mesenchymal and endothelial cells, are critical to maintain LN integrity and function, playing a key role in LN organogenesis, maintenance of LN cellularity and compartmentalization. Particularly, LNs are strategically situated proximal to lymphatic and vascular branching points to effectively filter antigens and facilitate immune cell entry from the surrounding tissues [13]. Myeloid cells are organized in a complex network of immune cells that play a key role in both innate and adaptive immunity, in the removal of dying cells and in tissue remodeling. Myeloid cells develop from multipotent hematopoietic stem cells that progressively differentiate into more specialized and restricted progenitor cell types leading to the granulocytic lineage or the monocytic lineage. The later differentiates into monocytes and finally to macrophages and dendritic cell subsets. Macrophages exhibit profound plasticity that enables them to differentiate into various phenotypes depending on signals from their local microenvironment [14]. Classically, activated macrophages (M1 macrophages) mediate host defense against a

variety of bacteria, protozoa and viruses, and play a role in antitumor immunity. Alternatively activated macrophages (M2 macrophages) show anti-inflammatory function important for resolution and regulate wound healing [15].

In conclusion, both synovium and hematopoietic organs can exert a comprehensive niching function for the development of their cell components. Further studies are required to better delineate these nurturing and protective functions.

The “Evil” or the Detrimental Side

Nurse-Like cells and fibroblasts-like synoviocytes in joint pathologies

Altered cellular composition and gene expression in the synovium is associated with joint pathologies, notably RA and osteoarthritis (OA). RA is characterized by a chronic inflammation with infiltration of a variety of inflammatory cells, including T and B-cells as well as those of myeloid origin into the affected synovium. One important feature of rheumatoid inflammation is the local B-cell activation, resulting in the production of large amounts of autoantibodies. Based on the hypothesis that pathological cells present in RA could originate from the synovium or the bone marrow and then migrate into the joint space, different groups focused their studies on fibroblastic stromal cells derived from synovium and bone marrow of RA patients with conditioned medium. Among these cells, a population exhibiting nurse-like cells functions was identified. These RA-NLCs had the capacity to carry out pseudo-emperipoiesis, since they formed unique complexes with various lymphocytes which could migrate among them without being internalized. They promoted survival and enhanced function of not only T cells but also B cells and secreted large amounts of cytokines, notably IL-6, IL-8, GM-CSF, IL-1 β and TNF α . Particularly, activation of RA-NLCs and B-cells enhanced survival and differentiation of B-cells and fostered proinflammatory activity of RA-NLCs by increasing their cytokine production [16-18]. Also, RA-NLCs directly interact with monocytes, promoting their maturation and differentiation into multinucleated osteoclast-like cells in a characteristic two-steps process. These cells have the capacity to degrade bone and cartilage [19]. RA-NLC phenotype argues for a mesenchymal origin, since they express CD29, CD44, CD49c, CD54, CD106, and class I major histocompatibility complex molecules, but do not express CD1a, CD18, CD35, CD40, CD154, or CD56 [16]. Particularly, B-cell apoptosis is inhibited by the engagement of CD49d/CD29 expressed on B-cells with CD106-expressing NLCs, which results in the upregulation of anti-apoptotic Bcl-x_L in B-cells [20]. Indeed, constitutive expression of CD106, even upon long-term culture in absence of cytokine stimulation, appeared as a characteristic feature of RA-NLC, distinguishing them from fibroblasts. Moreover, they were positive for vimentin, which is characteristically expressed in cell of mesenchymal origin [21].

Somehow controversial, Burger *et al.* showed that ordinary fibroblasts-like synoviocytes obtained from RA and osteoarthritis (OA) patients may support B-cell pseudo-emperipoiesis *via* a mechanism dependent on the expression of SDF-1 and CD106. Notably, IL-4-induced expression of CD106 allowed dermal fibroblasts to support B-cell pseudo-emperipoiesis, highlighting the role of CD106 [22]. These results were confirmed by other groups [23-25]. The minor population of RA-NLCs in an RA joint could facilitate the local accumulation, proliferation, and activation of

B- cells and would be implicated in not only the initiation of an inflammatory response but also during the absence of resolution typical to RA joint inflammation. Their absence in OA joint could explain the lack of inflammation. On the other hand, if all fibroblasts were capable to support B-cell pseudo-emperipolesis, all joints would be susceptible to chronic inflammation. In this context, the enhanced microvascular permeability caused by the inflammatory effectors allows immune competent cells from the circulation to enter the joint and settle in the nurturing environment provided by FLSs. This could explain the differences between chronic inflammatory and non-inflammatory forms of arthritis, like OA, in which an insufficient number of circulating cells enter the synovium [22]. Whichever origin of supporting cells is prevalent, the intriguing finding of these studies is that the nurturing capacity of certain cells to physiologically support other ones might be turned to a detrimental effect inducing and maintaining pathological conditions (Figure 2). As reported above, in normal joints, mesenchymal stem cells, including BM-MSCs from which RA-NLC originate, have a beneficial role, through the maintenance of joint homeostasis and repair of small lesions.

Dr. Jekyll becomes Mr. Hyde: which is the poisoned potion responsible for this unsolicited transformation?

Since stem cell differentiation appears disrupted in human inflammatory rheumatic diseases [26], modified BM-MSCs or their progeny could directly worsen arthritis. Increased migration of BM-MSCs in the synovium combined with local inflammation could fuel excessive immune responses. Nevertheless, the ectopic

presence of BM-MSCs in the synovium is probably not sufficient to induce long lasting immune responses, and some acquired intrinsic changes in BM-MSCs within the synovium could be required to sustain chronic inflammation [27,28]. Several studies suggest that some BM-MSCs might behave abnormally and/or are epigenetically modified in RA, following exogenous triggers, or metabolic changes [11,29-31]. Likewise, serum and/or proinflammatory cytokines, particularly IL-4 can induce phenotypic changes in fibroblasts that allow for B-cell pseudo-emperipolesis and survival suggesting that inflammatory cytokines can act to enhance the nurse-like function of normal fibroblasts [22].

Nurse-like cells: the tumor associated M2-type macrophages (TAM) in chronic lymphocytic leukemia (CLL)

Deregulation of hematopoiesis is associated with the development of hematological malignancies. Emerging evidences show that the BM microenvironment, with its complex and dynamic network of distinct cell types supporting hematopoiesis, plays a central role in the initiation, maintenance and outcome of myeloid disorders. As well, in the BM and secondary lymphoid organs, chronic lymphocytic leukemia (CLL) cells undertake complex, albeit incompletely defined so far, cellular and molecular interactions, with stromal cells, immune cells and matrix, collectively referred to as the “tumor microenvironment.” This interplay makes pivotal contribution to disease progression, as extensively described in several previous reviews [30-33]. Physiological hematopoiesis, as mentioned above, depends on BM stromal cells (BMSCs), which provide attachment sites and growth factors to hematopoietic precursors.

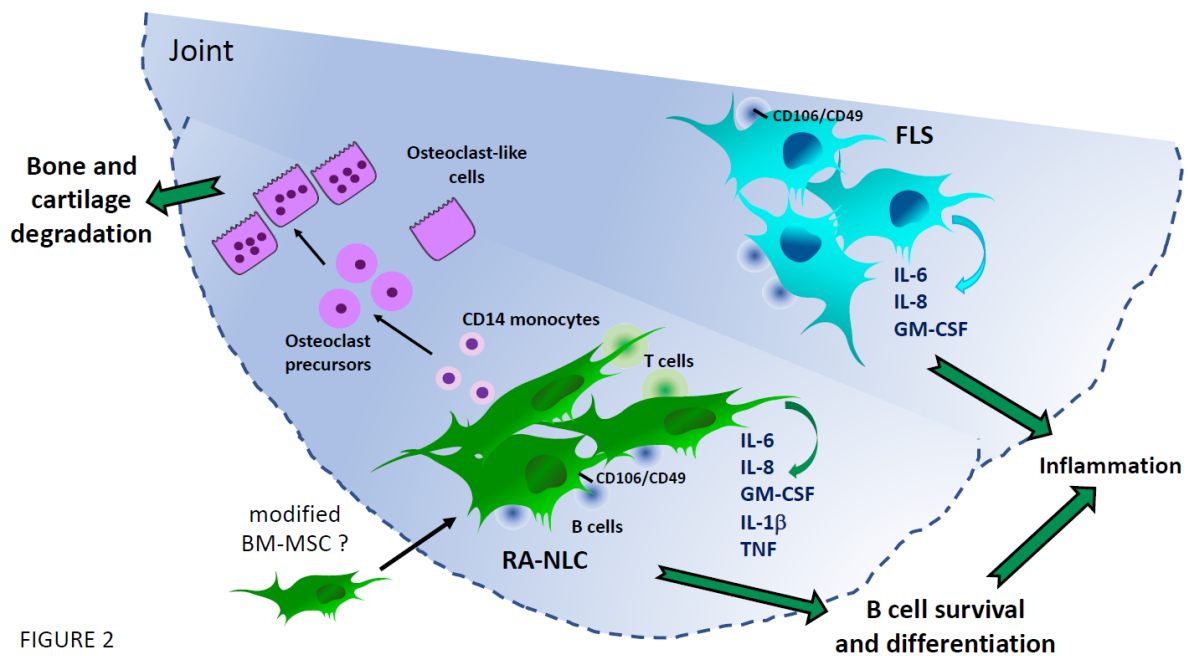


Figure 2. Role of RA-NLCs and FLS in supporting inflammation in joint pathologies. Epigenetically modified BM-MSC migrate to the synovium where they assume nurse-like cell functions (RA-NLC) and foster inflammation. They interact with T and B cells supporting their survival and enhanced functions and secrete IL-6, IL-8, GM-CSF, IL-1b and TNF. B cells survival is dependent on the engagement of CD49d expressed on B cells with CD106 expressed on RA-NLCs. NLC can also directly interacts with monocytes, promoting their maturation and differentiation into multinucleated osteoclast-like cells which degrade bone and cartilage. Similarly, fibroblast-like synoviocytes support B cells survival through CD49d/CD106 interaction and secrete proinflammatory cytokines. BM-MSC: bone marrow mesenchymal stem cells. RA-NLC: Rheumatoid Arthritis Nurse-like Cell; FLS: Fibroblast -Like Synoviocytes.

Coculture of BMSCs with CLL cells results in the adhesion of the later. This process is dependent of CXCR4 and VLA-4 expression by leukemic cells offering them nutrients and protection from apoptosis and cytotoxic agents [34]. BMSCs are of mesenchymal origin and are counterparts of α SMA-positive mesenchymal stromal cells (MSCs) found in other tissues such as those present in secondary lymphoid organs. Dense infiltration of α SMA⁺ stromal cells in lymphoid tissues from CLL patients [35] suggests that their interaction with CLL cells plays an important role even outside of the BM. Contribution of myeloid cells to CLL progression was clearly demonstrated by Hanna *et al.* who showed that monocytes accumulate in all the organs affected by the disease in the E μ -TCL1 mouse model [36]. Accordingly, CD68^{high} CD163^{high} myeloid cells are found in the spleen and LNs of CLL patients in close contact with leukemic cells [37-39] as described in BM [40,41]. Burger *et al.* demonstrated that blood monocytes differentiate *in vitro*, under the influence of CLL cells, into large, round, adherent CD68⁺ cells that attract CLL cells and protect them from undergoing spontaneous or drug-induced cell death in a contact dependent manner. Since these cells showed common features with thymic nurse cells, they were designed as nurse-like cells (NLCs) [37,42]. Based on surface markers expression and similarity in gene expression profiles, NLCs were suggested to resemble tumor-associated, M2-type macrophages (TAM) [43,44]. TAMs are M2-like macrophages, which generate immune and non-immune responses in order to promote various mechanisms. These macrophages promote angiogenesis, facilitate tumor cell invasion or metastasis and protect tumor cells from chemotherapy-induced apoptosis [45]. Moreover, NLCs exhibit immunosuppressive functions attributed to M2-like TAM by supporting Foxp3⁺ regulatory T cells (T_{reg}) expansion and by inhibiting helper T-cell proliferation [46]

NLCs prevent CLL cells from apoptosis *in vitro* notably through the expression of BAFF or APRIL [47], CD31, plexin-B1, Brain-Derived Neurotrophic Factor (BDNF) [48,49] and the secretion of soluble CXCL12. The engagement of CXCR4 on CLL cells by CXCL12 induces the activation of downstream signaling pathways such as MAP kinase and AKT, two crucial effectors in maintaining CLL cells survival [50].

NLCs play also a critical role in CLL B-cells trafficking. Notably, CXCL12 is a potent chemoattractant for CLL cells and behaves as an important player in the recirculation of plasma cells and homing in BM niches [51,52] as well as in the trans-endothelial migration of CLL cells [53]. In addition, NLCs secrete CXCL13, a homeostatic chemokine normally expressed by follicular dendritic cells (FDCs). CXCL13 interacting with its cognate receptor CXCR5 is responsible for lymphocytes homing in secondary lymphoid organs and for active trafficking within the follicles [54]. Leukemic cells express high levels of functional CXCR5 and, significantly increased CXCL13 serum levels were described in CLL patients, as compared to healthy controls. Thus, CXCL13 expressed by NLCs plays an important role in CLL cells niching [39]. We recently described that CD68⁺NLCs differentiated *in vitro* are a source of CCL21 homeostatic cytokine, which is retained at the cell membrane, providing a potent chemoattractant signal for CLL cells. We observed similar CD68⁺ cells in lymph node sections, which were able to express CCL21. The significant increase of CD68⁺ cells in lymph nodes from CLL patients paralleled a loss of FRC network organization. This result presents CD68⁺ cells as an additional and alternative source of CCL21 supplanting the role attributed to FRCs [55]. CCL21 plays a pivotal

role in driving B-cells entry and motility in interstitial lymph nodes favoring their interaction with CD4⁺ T cells expressing CD40L [56]. Importantly, trans-endothelial cell migration (TEM) in response to CCL21 was found significantly increased for malignant B-cells from patients presenting lymph node enlargement. Furthermore, the level of expression of CCR7 correlated with lymphadenopathy and its blockade inhibited CLL TEM [57]. In this context, CCL21 retained on NLCs likely plays a critical role in capturing malignant B-cells thus favoring their niching and survival and allowing immune tumor survey evasion and resistance to treatment [55].

Although the role of NLCs in supporting CLL cell survival is well documented, the mechanism of their differentiation remains largely elusive. Noteworthy, NLCs cannot derive from every single CLL patient blood sample. Moreover, when NLCs are generated, both the kinetic and the yield of their production are highly variable. No significant correlation was observed between the proportion of NLCs and the stage of the disease according to Rai classification. Nor were associated the expression of markers at risk of progression such as ZAP70 or CD38 [58-59]. Several evidences show that a crosstalk between NLCs and CLL cells creates an immunosuppressive milieu allowing immune escape. Borrowing again Stevenson's character, CLL B-cells are good friends of Mr. Hyde. Coculture of monocytes isolated from healthy donors with CLL cells induces the former to differentiate into cells that are indistinguishable from the NLCs derived from blood mononuclear cells of patients with CLL [37]. NLC coculture induced BCR activation and production of two T cell - chemoattractants, namely CCL3 and CCL4, by CLL cells [60]. Similarly, Schulz *et al.* showed that CLL cells actively induce an inflammatory milieu by enhancing the secretion of CCL2 and other cytokines from monocytes which further results in the recruitment and activation of myeloid cells and other immune cells [61]. Importantly, high levels of CCL3 and CCL4 in the CLL environment have been shown to attract monocytes *via* signaling through CCR1 and CCR5 [62]. Other soluble molecules produced by CLL B-cells, such as HGMB1 or CSF-1, are involved in monocytes to NLCs differentiation [63-64]. Tumor cell-derived exosomes are likely involved in the cross-talk between B-cells and monocytes. Haderk *et al.* demonstrated that CLL-derived exosomes and their non coding Y RNA, were uptaken by monocytes and macrophages and induced the secretion of inflammatory cytokines as well as PD-L1 expression thus contributing to a supportive tumor microenvironment [65]. Recently, Domagala *et al.* showed that IL-10 might reprogram NLC cells towards an M2-like supportive phenotype leading to increased leukemic cells survival. On the opposite, TNF α was able to depolarize M2-type protective NLC towards M1-type, decreasing CLL cell survival. The molecular balance between these two cytokines, both produced in certain circumstances by CLL B-cells, could play an important role in the maintenance of the protective NLC phenotype in CLL niches [66]. Thus, the tumor niche constitutes a self-sustaining immune suppressive environment in which leukemic cells favor an increased number of differentiated M2-like NLCs/TAMs, which in turn provide CLL pro-survival factors as well as suppression of anti-tumor immunity within the CLL microenvironment (**Figure 3**).

Accordingly, M2-polarization of NLC cells characterized by high CD163 staining in lymph nodes was correlated with worse prognosis in CLL [38]. Recently, in Richter syndrome, a transformation of CLL into an aggressive lymphoma, a higher infiltration of CD163-positive macrophages was observed in nodal tissue as compared to CLL [67].

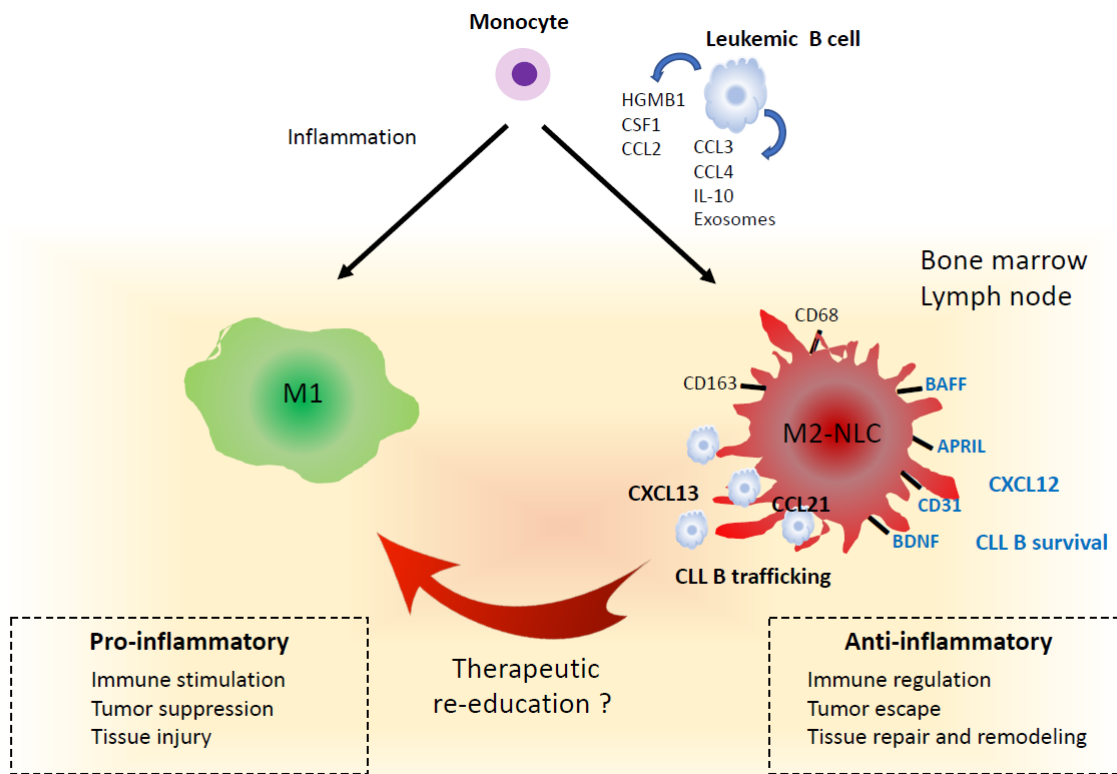


Figure 3. Nurse-like cells: the tumor associated M2-type macrophages (TAM) in chronic lymphocytic leukemia (CLL). Chemo-attractants secreted by leukemic B cells recruit newly matured monocyte-derived macrophages to the CLL niche in bone marrow and lymph nodes where they acquire the M2-like phenotype. Differentiation to the M2-phenotype is driven by HGMB1, CSF1 and IL-10 or exosomes produced by leukemic B cells. NLCs provide pro-survival signals for CLLs through the expression of BAFF, APRIL, CD31 and BDNF and the secretion of CXCL12. NLCs secrete CXCL13 which plays a role in B cells trafficking. NLCs produce also CCL21 which is retained at the cell membrane and provides a potent chemoattractant signal for CLL cells.

Alternatively, inflammation signals polarize macrophages towards M1 phenotype which exerts antitumoral activity. A promising approach to therapeutic targeting of the tumor microenvironment aims to re-program M2 macrophages towards M1-phenotype. CLL: Chronic Lymphocytic Leukemia.

Similarly, a higher proportion of CD68⁺CD163⁺ macrophages was associated with poor prognosis in cutaneous melanoma [68] and in human or murine sarcoma [69]. In our study, we observed that CD68⁺ NLCs obtained from various patients showed heterogeneous proportions of CD163⁺ cells. Interestingly, when we analyzed the presence of CCL21 mRNA and protein, we reproducibly observed a subset of CD163⁺ cells expressing only CCL21 mRNA, which suggests different levels of differentiation for these cells [55].

In addition to their roles in tumor tolerance NLCs might be implied in distinct functions between physiological or pathological contexts. Indeed, Bhattacharya *et al.* demonstrated the presence of CD68⁺ cells in non-malignant tonsil and lymph node sections from healthy individuals raising the possibility that NLC-like cells are present in the lymphoid tissues of healthy individuals as well as CLL patients [70]. However, these “healthy” NLCs express very low levels of CD163, do not produce CCL4 and are unable to promote survival of CLL cells [71]. Similarly, we detected CD68⁺ cells in non-tumoral lymph nodes. These cells were positive for CCL21 staining, although to a lesser extent than the CD68⁺ cells present in CLL lymph nodes. Importantly, these non-tumoral samples were issued

from individuals presenting with different degrees of inflammatory process while in absence of malignant progression [55]. Carlsen *et al.* showed that human CD68⁺ differentiated monocytes are potent inducible CXCL13 producers, another specific NLC effector. These macrophages represented the main source of the chemokine in inflammatory lesions where lymphoid neogenesis occurred [72]. These findings suggest that NLCs could indeed have different roles according to their anatomical positioning, favoring tumor cell survival and retention or, alternatively, recruiting cells during an inflammatory reaction.

Understand the Opposite Beneficial/detrimental Behaviors to Target and Re-educate NLCs

Increasing evidences about the critical role of a deviant microenvironment in the initiation and maintenance of pathological conditions suggest to interfere with its protective functions in a therapeutic approach. This goal calls for an improved understanding of the detrimental functions of microenvironmental cells.

Notably, the tumor microenvironment is immunosuppressive and pro-tumorigenic whereas an inflammatory microenvironment,

exemplified here by the arthritic synovium, is pro-inflammatory and against resolution. Despite their opposing immunological status, they exhibit comparable metabolic status: both are hypoxic and, show elevated levels of metabolites, such as lactate, and low levels of nutrients. These observations suggest that an altered availability of metabolites might contribute to a dysregulated immunological status [73] and to detrimental functions of the microenvironment cells.

BM-MSCs are considered as appealing therapeutic candidates for inflammatory and rheumatic diseases due to their immunomodulating properties and regenerative potential [26]. Conversely, the abnormal behavior, likely due to acquired defects, of some BM-MSCs and/or of their progeny might represent an underrated issue fueling pathological conditions. An actual working hypothesis proposes that experienced BM-MSCs induced by transient trafficking of dead or live pathogens, in subchondral BM close to joint and/or entheses, contribute to this unusual behavior and to the pathogenesis of inflammatory rheumatism. These evidences underline the need for a deeper characterization of stem cells in synovium but also subchondral BM and enthesis BM [74].

In CLL, the actual therapeutic strategies focus majorly toward the leukemic cells. However, there is also, an increasing interest in targeting the nurturing capacity of the microenvironmental cells [75,76].

Very interestingly, specific therapeutic agents can modulate nurse-like cells function by “re-educating” them. Particularly, Fiorcari *et al.* demonstrated that lenalidomide, an immunomodulatory agent used during CLL therapy, reduces the production by NLCs of pivotal pro-survival factors. The therapeutic agent also supports an orientation towards macrophage M1 phenotype and improves the phagocytic activity as well as the ability to activate T-cell proliferation. Moreover, lenalidomide modifies CLL-circulating monocytes, inducing a loss of migration through modulation of small GTPases and their firm adhesion to the endothelium. These evidences suggest that lenalidomide mediates a pro-inflammatory switch of the nurse-like cells affecting the protective microenvironment in the tissue [77]. Recently, Merchand-Reyes *et al.* showed that specific inhibition of the MEK pathway by trametinib dramatically reduced NLC development *in vitro*, affecting indirectly CLL cells survival. Using the E μ -TCL1 adoptive transfer mouse model, they could extend their findings *in vivo* showing that MEK inhibition results in fewer splenic monocytes/macrophages, preferentially affecting the M2-like populations [78].

The effects of ibrutinib, the BTK inhibitor widely used in CLL therapy, on NLCs are more controversial. We and others observed that ibrutinib treatment does not alter the capacity of CLL to induce monocyte differentiation toward NLC phenotype. NLCs maintained their capacity to preserve CLL cells from *ex vivo* spontaneous apoptosis [55,79,80]. Noteworthy, Fiorcari *et al.* showed that ibrutinib further misled NLCs, supporting their nurturing and protective behavior by potentiating their M2-like immunosuppressive profile and by leading to the secretion of unwanted survival factors [80]. Correspondingly, in our co-culture experiments, ibrutinib exposure did not impact the secretion of pro-survival CXCL12 by NLCs nor the expression of CXCR4 on CLL B-cells maintaining thereby a protective milieu [55]. Nevertheless, Nieuemann *et al.* showed that, in the BM microenvironment,

ibrutinib disrupted the interactions between macrophages and CLL cells, inhibited the secretion of CXCL13 and decreased the chemoattraction of CLL cells [41]. Accordingly, we detected reduced B-cells/NLCs interactions in our *in vitro* NLC model [55]. These evidences indicate that cotreatments with other anti-leukemic agents such as bendamustine or venetoclax, a Bcl-2 antagonist, could counteract the chemoprotective effect induced by NLC in ibrutinib-treated patients. Moreover, they suggest that unwanted ibrutinib resistance could arise from its lack of efficiency in blocking CLL-monocyte interactions, leaving NLC induction and pro-survival capacities unaffected and thus emphasizing the role of the TME in persistent residual disease [79]. In any case, they urge to further characterize interactions between CLL B-cells and NLCs.

NLCs were also described for mediating resistance to venetoclax, the Bcl2 antagonist and Bcl2 being highly expressed in CLL cells, through an aberrant expression of Wnt5a. Neutralizing Wnt5a significantly inhibited NLC-induced survival, migration and the corresponding cell signaling in CLL cells, indicating novel mechanisms for venetoclax resistance as well as potential therapeutic options to circumvent this resistance [81].

Conclusion

Nurse-like cells are intriguing and challenging players of the aberrant transformations of the microenvironment creating a permissive milieu for the progression of chronic inflammatory diseases and malignant hematopoiesis (Figure 4). In this review, we focused on nurse like cells in CLL but tumor microenvironment actively contributes to the progression of several leukemias and lymphomas as well as of solid tumors. Moreover, other stromal cells, particularly tumor-associated neutrophils (TANs), myeloid-derived suppressor cells (MDSCs) and tumor associated dendritic-cells (TADCs) critically contribute to the suppression of innate and adaptive antitumor immune responses. The translational relevance of targeting this complex network is obviously very high. However, the characterization of the cellular composition and the origin of the various cell types present in the microenvironment as well as the elucidation of underlying molecular mechanisms of their crosstalks are critical to design new therapeutic strategies. Whereas normal BM-MSCs are expected to be a solution for various human disorders, the dangerous behavior of subsets of subchondral BM-MSCs, which might conversely fuel inflammation has to be carefully considered. Therapeutic targeting of the TME to reinstall protective antitumor immunity through interference with the recruitment of myeloid cells, repolarization of immune cell subsets and inhibition of lymphoma promoting signals is a promising strategy. Nevertheless, whereas the critical role of myeloid cells is well established, it remains a delicate balance to achieve a repolarization rather than a depletion of the pro-tumorigenic innate immune cell population. Moreover, it becomes increasingly uncertain which subset or differentiation stage is better to choose as a target, accordingly to results describing highly complex myeloid differentiation states in lung tumors [82].

These observations highlight the need to fully characterize the cellular composition of the permissive niches and define whether the immunosuppressive and tumor-sustaining function are dependent on their maturation and activation status.

As for M. Utterson, Dr. Jekyll's friend, the mystery is still long to be understood.

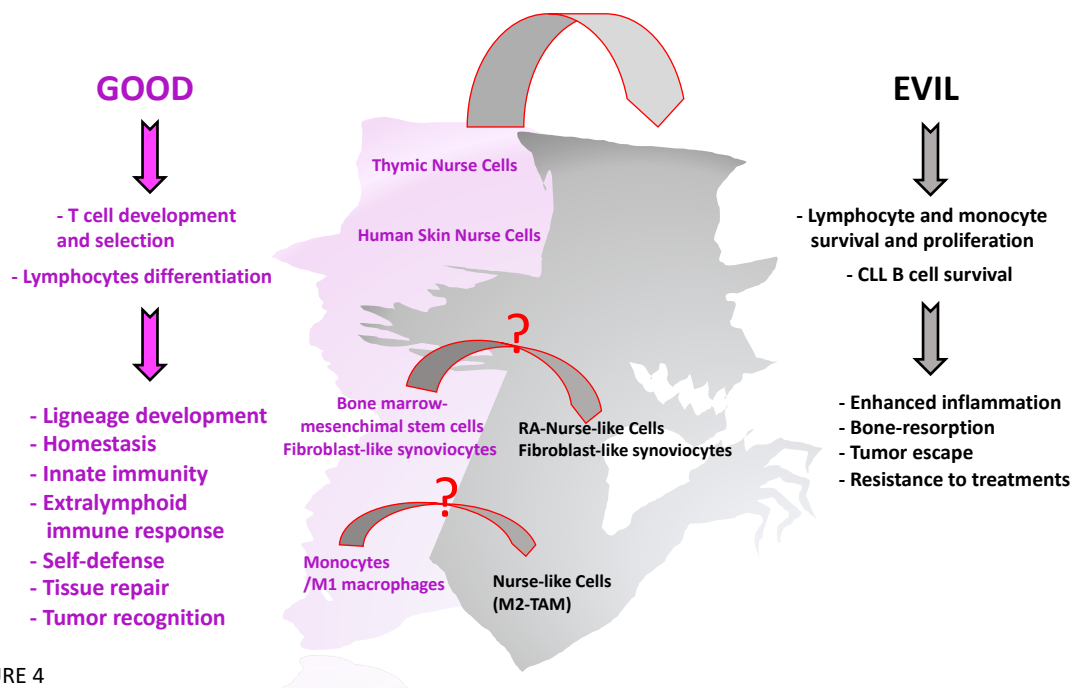


FIGURE 4

Figure 4. The good and the evil sides of Nurse-like cells and their effects. In the left side, the “good” nurse cells and their nurturing and patrolling functions in supporting different processes are shown in magenta. In the right side, the “bad” nurse cells and their detrimental functions, which fuel pathological conditions are shown in grey.

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